GENETICS

A periodical record of investigations bearing on heredity and variation

Founded in 1916 by GEORGE H. SHULL

with the cooperation of

WILLIAM E. CASTLE
EDWIN G. CONKLIN
CHARLES B. DAVENPORT
BRADLEY M. DAVIS
EDWARD M. EAST
ROLLINS A. EMERSON
HERBERT S. JENNINGS
THOMAS H. MORGAN
RAYMOND PEARL

VOLUME 45 FEBRUARY, 1960 NUMBER 2

TABLE OF CONTENTS

PARKER, D. R., The induction of recessive lethals in Drosophila oocytes . 135

GOWEN, JOHN W., A Drosophila intersex-triploid 139

KREIZINGER, JEAN D., Diepoxybutane as a chemical mutagen in *Zea mays* 143

HAYMAN, B. I., The theory and analysis of diallel crosses. III. 155

SCHEINBERG, S. L., Genetic Studies of serum antigens in species hybrids . 173

OGUR, M., S. OGUR, and R. ST. JOHN, Temperature dependence of the spontaneous mutation rate to respiration deficiency in Saccharomyces 189

MAGUIRE, MARJORIE P., A study of homology between a terminal portion of Zea chromosome 2 and a segment derived from Tripsacum 195

HUBBY, J. L., and H. S. FORREST, Studies on the mutant maroon-like in *Drosophila melanogaster* 211

KIDWELL, J. F., H. J. WEETH, W. R. HARVEY, L. H. HAVERLAND, C. E. SHELBY, and R. T. CLARK, Heterosis in crosses of inbred lines of rats . 225

STIMPFLING, J. H., and M. R. IRWIN, Gene homologies in Columbidae . . 233

PUBLISHED MONTHLY AT AUSTIN, TEXAS
BY GENETICS, INC.

(Date of issue, May 5, 1960)

EDITORIAL BOARD

CLARENCE P. OLIVER, *Managing Editor*,
WILSON S. STONE, *Managing Editor*,
University of Texas

GEORGE W. BEADLE
California Institute of Technology

R. ALEXANDER BRINK
University of Wisconsin

WILLIAM E. CASTLE
University of California

JAMES F. CROW
University of Wisconsin

EVERETT R. DEMPSTER
University of California

L. C. DUNN
Columbia University

JOHN W. GOWEN
Iowa State College

A. D. HERSHEY
Carnegie Institution of Washington

DONALD F. JONES
Connecticut Agricultural Experiment Station

M. M. RHOADES
Indiana University

T. M. SONNEBORN
Indiana University (Representative of the Genetics Society of America)

CURT STERN
University of California

ALFRED H. STURTEVANT
California Institute of Technology

SEWALL WRIGHT
University of Wisconsin

VOLUME 45 FEBRUARY, 1960 NUMBER 2

GENETICS is a monthly journal issued in annual volumes of about 1000 pages each.

Subscription, $12.00 net a year for complete volumes (January–December). Foreign postage, $1.00 additional. Single copies, $1.50 each, postpaid.

As available, back volumes 1 through 44 may be had at $14.00 each and single issues of previous volumes at $2.50 per copy, postpaid. The Business Office will supply information on request as to volumes and numbers available.

Business Correspondence, including change of address, etc., should be addressed to GENETICS, INC., Business Office, Experimental Science Bldg. 122, University of Texas, Austin 12, Texas.

Remittances should be made payable to **GENETICS, INC.**

Correspondence concerning editorial matters should be addressed to the EDITORS OF GENETICS, Experimental Science Building 122, University of Texas, Austin 12, Texas. 3579 should be sent to this address also.

Second-class mail privileges authorized at Austin, Texas.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

Expansion of this issue to include extra papers has been made possible by a National Science Foundation Grant-G7194 which was given in part for support of publication of manuscript backlog. The Editors express appreciation for the support.

THE INDUCTION OF RECESSIVE LETHALS IN DROSOPHILA OOCYTES[1]

D. R. PARKER

Division of Life Sciences, University of California, Riverside

Received November 20, 1959

WORK on recessive lethal mutation in Drosophila sperm has suggested ways in which chromosome breakage may be related to recessive lethal mutation, through position effect, deficiency, and as a direct result of breakage.

The increase in aged oocytes of radiation-induced dominant lethals (PARKER 1959) and of rearrangement, the slower rejoining of breaks (PARKER and HAMMOND 1958), and the higher yield of deficiency and of exchange between attached-X and a major autosome (PARKER and MCCRONE 1958), suggest that the effects of the aging of these cells on the induction of recessive lethals by irradiation might throw some light on the possible relation of their induction to breakage and rejoining. The probability that a break will restitute, rejoin, or fail to rejoin may differ considerably in different stages.

EXPERIMENTAL PROCEDURE

Irradiation was at 100 KVP, 5 ma, with a 1 mm Aluminum filter, and a dose rate of 300r/minute.

Drosophila melanogaster oocytes in stages 7 or 14 were irradiated. Virgin, wild type (Oregon-R) females were divided into appropriate groups for irradiation: those to be irradiated immediately and those to be aged before treatment. Females were treated either 0–12 hours after emergence (stage 7) or after aging five days (stage 14); the former were held for one (series 1) or two days (series 2) before being mated, while the latter were mated immediately after being irradiated. Females were mass-mated with Muller-5 (sc^{s1} *B In S* w^a sc^s) males (series 1, only) or with males of a modified Muller-5 stock carrying a lethal allele of bobbed, and were allowed to lay eggs for 24 hours. All F_1 females emerging were collected virgin and backcrossed to the standard or the modified Muller-5 stock males. A culture was scored as lethal if no wild type males were included among 30 or more offspring in series 1, 20 or more in series 2. Where fewer offspring were produced, with no wild type males, heterozygous females were backcrossed for another generation.

RESULTS

The data of these experiments are summarized in Table 1. In series 1, using stage 7 only, the control value was found to be excessive. The Oregon-R stock

[1] This work was supported by contract AT-(40-1)-2163 of the U. S. Atomic Energy Commission with the University of Mississippi.

Second Printing 1966 / University of Texas Printing Division, Austin

TABLE 1

Induction of recessive lethals

Series	Stage treated	Dose, r	No. tested chromosomes	No. lethal	Percentage lethal	Percentage induced/kr
1	7	0	2,095	19	0.91 ± .21	...
1	7	1,000	2,665	48	1.80 ± .26	0.89
1	7	2,000	2,035	64	3.14 ± .39	1.12
1	7	3,000	1,045	58	5.55 ± .71	1.55
1	7	4,000	756	55	7.28 ± .94	1.59
2	7	0	3,286	3	0.09 ± .05	...
2	7	250	3,027	9	0.30 ± .10	0.84
2	7	500	2,593	14	0.54 ± .14	0.90
2	7	1,000	2,353	27	1.15 ± .22	1.06
2	7	2,000	1,551	39	2.51 ± .40	1.21
2	7	3,000	997	39	3.91 ± .61	1.27
2	7	4,000	887	38	4.28 ± .68	1.05
2	14	0	3,836	0	0	...
2	14	125	2,650	12	0.45 ± .13	3.60
2	14	250	2,236	11	0.49 ± .15	1.96
2	14	500	1,188	13	1.09 ± .30	2.18
2	14	750	644	16	2.48 ± .61	3.30

was freed of lethals, and irradiations were repeated with both stages 7 and 14 being used. With two exceptions, it was found that the "percentage induced per kr" increased with increasing dosage, as would be expected if there is a "two hit" component in the lethals in both stages. Data were fitted to linear and to second degree curves, with the following results:

Series 1:

$y = 0.47 + 1.57\,D$ ($x^2 = 11.05$, 3 d. f.)

$= 0.86 + 0.74\,D + 0.23\,D^2$ ($x^2 = 0.519$, 2 d. f.)

Series 2, stage 7:

$y = 0.04 + 1.16\,D$ ($x^2 = 1.25$, 5 d. f.)

(*The coefficient of* D^2 *is negligible in the second-degree equation.*)

Series 2, stage 14:

$y = -0.02 + 2.76\,D$ ($x^2 = 4.48$, 2 d.f.)

$= 0.07 + 1.24\,D + 2.37\,D^2$ ($x^2 = 0.935$, 1 d. f.)

(D = dose, measured in kiloroentgens)

DISCUSSION

Evidence for multihit induction of some recessive lethals has been found by EDINGTON (1957) who showed that a quadratic equation fit better than a linear one for frequency of X-ray lethals induced in Drosophila sperm. LINDSLEY, EDINGTON and VON HALLE (1958) found a number of position-effect lethals in

irradiated Drosophila sperm. HERSKOWITZ and ABRAHAMSON (1955) showed the frequency of recessive lethals in oocytes to be less when a less intense dose of radiation was used, and concluded that a large number were "multihit".

For a single set of data, a quadratic should fit better than a linear regression as a result only of chance deviations from a straight line. However, the coefficient of the squared term should as often be negative as positive, and adequate replication of the experiment should show this. Two factors work in favor of a negative coefficient for D^2: first, a "linear" relation would most likely be $y = 1 - e^{-kD}$, and second, heterogeneity of the sample.

Despite precautions, there will be some heterogeneity of sensitivity of cells in any sample. This has been commented on for dominant lethals (PARKER 1959). Samples of stage 14 are contaminated with a few cells similar to stage 7. An estimate of six percent of such contamination would lead to nearly 20 percent contamination among the survivors at 750r. With fewer lethals occurring in the contaminating cells, progressively larger underestimates will be made as dosage increases. Similar contaminations of stage 7 samples may occur.

Collectively, the evidence for "two hit" recessive lethals seems convincing, and the higher incidence of lethals in stage 14 could be due largely to these. More small deficiencies and position effect lethals might be predicted from the finding of many more deficiencies and exchanges with non-homologous chromosomes in stage 14.

A comparison of doses producing equivalent damage in stage 7 to that produced by a given dose, 500r, in stage 14 is instructive. For dominant lethals, 5500r is required (PARKER 1959); for detaching attached-X, 2000r (PARKER and HAMMOND 1958); for recessive lethals, 1000r. Many dominant lethals induced in stage 14 may result from failure of breaks to rejoin, and are "one hit". The increase in stage 14 of rearrangements (detachments), with a higher rate of loss of breaks, means either nearly all breaks restitute in stage 7, and/or there is a higher frequency of breakage in stage 14. Were "one hit" lethals related to breakage, and were there no more breaks induced in stage 14 than in stage 7, one should find a reduction of these in stage 14 because of failure of breaks to restitute or rejoin eucentrically. An increase in frequency of "one hit" lethals in stage 14 would require greater primary damage in that stage, damage that might or might not be breakage of chromosomes.

The results given here cannot resolve the questions that have been raised; their resolution will require the demonstration of such qualitative differences as may exist between lethals induced in the two stages, the collection of more data on the relative frequencies in the two stages of chromosomal aberrations (especially deficiencies), and more data on the effects of treatments known to modify dominant lethal production (via changing probability of restitution?) on the production of recessive lethals in stage 7. The resolution of these questions is made difficult by our concern with two sets of uncertainties: the nature of the sensitivity changes in primary oocytes, and the nature of the processes giving rise to recessive lethals.

SUMMARY

Recessive lethal induction by X-irradiation of stages 7 and 14 of primary oocytes of *Drosophila melanogaster* was studied. Stage 14 gives about a twofold increase in lethals over stage 7. There is evidence for the occurrence of some "two hit" lethals. Speculation is made on possible relations between sensitivity changes, breakage and rejoining, and recessive lethals.

LITERATURE CITED

EDINGTON, C. W., 1957 The induction of recessive lethals in *Drosophila melanogaster* by radiations of different ion density. Genetics **41**: 814–821.

HERSKOWITZ, I. H., and S. ABRAHAMSON, 1955 The effect of X-ray intensity on the rate of sex-linked recessive lethal mutation induced following treatment of Drosophila oocytes. Drosophila Inform. Serv. **29**: 125.

LINDSLEY, D. L., C. W. EDINGTON, and E. S. VON HALLE, 1958 Sex-linked recessive lethal changes in *Drosophila melanogaster* suppressed by the Y chromosome. Proc. 10th Intern. Congr. Genet. (Montreal) **2**: 172–173. (Abstr.)

PARKER, D. R., 1959 Dominant lethal mutation in irradiated oocytes. *Biological Contributions.* Univ. Texas Publ. **5914**: 113–127.

PARKER, D. R., and A. E. HAMMOND, 1958 The production of translocations in Drosophila oocytes. Genetics **43**: 92–100.

PARKER, D. R., and J. MCCRONE, 1958 A genetic analysis of some rearrangements induced in oocytes of Drosophila. Genetics **43**: 172–186.

A DROSOPHILA INTERSEX-TRIPLOID[1]

JOHN W. GOWEN

Department of Genetics, Iowa State College, Ames, Iowa

Received January 4, 1960

GYNANDROMORPHS and similar mosaics in Drosophila have furnished information on the sufficiency of a gene, chromosome or genome to control its phenotype when in cell-to-cell proximity with cells of other gene constitutions. Generally gene or chromosome actions appear limited to the cell in which they are contained. The exceptions appear in vermilion-like-genes which generate products influencing other cell phenotypes. Gynandromorphs which result from the loss of an X chromosome from one of the daughter cells of an early division of an XX type cell, designed to form the left or right side of the body, show distinct male characteristics on that side without influence on the female characteristics on the other side. This and other evidence indicates that, for Drosophila, sex hormones of the types found in mammals may not be generated .This evidence is unsatisfactory on several grounds.[2] To some extent this may be due to the material which is used for the test. The male, X + 2A or XY + 2A, cells versus the female, 2X + 2A, cells are further apart in their gene balances than other cells with which Drosophila has familiarized us. The intersexes with their 2X + 3A or 2XY + 3A and triploids, 3X + 3A, offer cell forms which are in closer balance with regard to their gene content. The smaller difference between them should make them more susceptible to smaller differences in hormonal substances than gynandromorphs of the customary male and female patterns.

One representative of this new sex type appeared in the progeny of a recently established triploid stock derived through the use of our *C3G* gene. Photographs of this fly, together with that of its reproductive tract, are shown in Figure 1. The fly was divided by an anterior-posterior line which separated the halves into intersex on one side and triploid on the other.

The left side was intersex with a presumed constitution of 2X + 3A chromosomes. The body on this side was reduced in size from that of the normal triploid but was larger than that of the customary intersex. A sex comb was present on the left metatarsal joint as may be seen in Figure 1. This sex comb had teeth of characteristic size and length for intersexes but had only six teeth instead of 11, the customary number for intersexes, GOWEN 1931; GOWEN, FUNG and STADLER 1957; GOWEN and FUNG 1957. The eye and wing cells were large as character-

[1] Journal Paper No. J-3787 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 1180, 1187. This work has received assistance from Contract No. AT (11-1) 107 from the Atomic Energy Commission.

[2] Even in mammals hormonal influence on development of the sex organs is not clear. In man, and particularly in mice, (HOLLANDER, GOWEN and STADLER 1956) the primary and secondary reproductive organs of each sex may exist together in the same animal.

FIGURE 1.—Photographs of an intersex-triploid Drosophila.

istic of intersexes. The wing on the left side was reduced to 60 percent of the area of the triploid wing seen on the left side of the fly. The intersex wing was larger than wings of most intersexes. It was wrinkled as is frequently the case in intersexes.

The right side of the fly was triploid throughout. The body size was large as characteristic of triploids. The large size resulted in the twisting of the body toward the left side. The forelegs were typically triploid in character without sex combs. The eyes and wing cells were large, comparable with those of triploids. The wing area was fully as large as that of triploids.

The reproductive system was entirely female. Ovipositor was present. External genitalia were female throughout. On the left side the ovary was about half the size of the normal triploid ovary found on the right side. The left ovary was much larger than an intersex gonad with eggs at various stages of maturity as though it possibly was functional. The ovaries were attached to the entirely female reproductive duct.

After hatching the fly was mated but remained sterile for ten days. Progeny were then produced; 14 diploid females, 1 triploid female, 3 male type intersexes and 1 intermediate type intersex. No super males or super females were observed but this is not unexpected since they are quite nonviable under most conditions. It is a little surprising that no males were observed. The diploid females, as is often the case in triploid matings were the most numerous class. The intersexes should equal the diploid females but seldom do. However, they were the next most numerous class. The triploid progeny bred as an ordinary triploid. The progeny of the intersex-triploid may all have come from eggs of the larger ovary on the triploid side of the fly although visually the eggs on either side appeared equally good.

The development of this intersex-triploid suggests some degree of hormonal-like interchange between the cells which developed as the right triploid side and the left intersex side of the fly. The reduction in number of teeth of the left sex comb, the decrease in wing and body size on the same side together with the egg development in the but half-sized left ovary all indicate an exchange of developmental substances between the cells of the fully formed triploid right side and the intersex halves of the body.

SUMMARY

A new Drosophila sex type consisting of left side intersex and right side triploid bred as a triploid. Left side was: somewhat larger than typical intersex, but smaller than triploid; sex comb had only six teeth; eye and wing cells large as in intersexes, but wing only 60 percent size of triploid wing; ovary developed to apparently fully formed eggs but was half the size of right triploid ovary; reproductive tract was female throughout. Right side was typically triploid. The organs on intersex side suggest that development was influenced by substances generated by the triploid half of the body.

LITERATURE CITED

GOWEN, JOHN W., 1931 Genetic nondisjunctional forms in Drosophila. Am. Naturalist **65:** 193–213.

GOWEN, JOHN W., SUI-TONG FUNG, and JANICE STADLER, 1957 Analyses of the contributions of the sex chromosomes, autosomes and a third chromosome gene series to sex comb development in Drosophila. Genetics **42:** 373.

GOWEN, JOHN W., and SUI-TONG FUNG, 1957 Determination of sex through genes in a major sex locus in *Drosophila melanogaster*. Heredity **11:** 397–402.

HOLLANDER, W. F., JOHN W. GOWEN, and JANICE STADLER, 1956 A study of 25 gynandromorphic mice of the Bagg albino strain. Anat. Record **124:** 223–243.

DIEPOXYBUTANE AS A CHEMICAL MUTAGEN IN ZEA MAYS[1]

JEAN D. KREIZINGER

Department of Plant Breeding, Cornell University, Ithaca, New York

Received April 6, 1959

DIEPOXYBUTANE (DEB) has been used very effectively as an inducer of gene mutations in Neurospora (KÖLMARK 1953; KÖLMARK and GILES 1953; KÖLMARK and WESTERGAARD 1953; WESTERGAARD 1957), chromosome deficiencies and rearrangements in Drosophila (BIRD and FAHMY 1953; FAHMY and BIRD 1953; FAHMY and FAHMY 1956), reversions in *Escherichia coli* (DEMEREC *et al.* 1954), and chromosome breaks in the *Vicia faba* root-tip test (LOVELESS 1951). In Neurospora, treatment with DEB was 20 times more effective on the induction of mutations at the adenine locus than were comparable doses of mustards, other epoxides, or ultraviolet. In Drosophila, DEB was found to be far less effective than X-rays in the production of major structural rearrangements in the chromosomes but considerably more efficient than X-rays in the production of small deficiencies. DEB affected the darker bands of the chromosomes preferentially although mustard gas affected the lighter ones, indicating a specificity of DEB for heterochromatin. This marked ability of DEB to induce gene mutations as well as chromosome breaks led to its being chosen for a study of chemically induced mutations in maize. The term "mutation", unless otherwise specified, is used here to include changes resulting from both chromosome breaks and so-called "point" mutations.

Maize has several characteristics which make it a very desirable object for studies of induced mutations. There are a number of endosperm characters determined by known genes of regular inheritance which are located in the short arm of chromosome 9. Maize endosperm, being triploid, is not adversely affected by considerable loss of genetic material. Chromosomes treated in mature pollen are in a prolonged resting stage and are not subject to the same stresses that exist during cell division. An F_1 generation can be observed for further test of the occurrence of point mutation or surviving chromosome abnormalities, if desired.

Although maize has been utilized extensively for studies of induced mutation by radiation, the use of chemicals for this purpose has been largely neglected. Nitrogen mustard treatments of mature pollen have been found very efficient as inducers of chromosome breaks observable in the endosperm (GIBSON, BRINK, and STAHMANN 1950; UMALI 1949). However, similar treatments with ethylene oxide were only moderately successful (FABERGÉ 1955). It is the purpose of the present paper to report on the effectiveness and specific characteristics of DEB as a chemical mutagen in maize.

[1] Published as Paper No. 367, Department of Plant Breeding, Cornell University.

MATERIALS AND METHODS

Genetic material

The standard procedure of utilizing multiple dominant stocks as the source of pollen for treatment and multiple recessive stocks as seed parents was followed. The absence of one or more dominant marker genes in the F_1 endosperm indicated loss of the markers involved. The word *loss*, designates only the disappearance of the phenotypic effect of the dominant allele and does not imply the particular mechanism involved.

The endosperm markers used in the following experiments are all located in the short arm of chromosome 9. They include the allelic color factors *I*, (inhibitor of aleurone color, dominant to *C*), *C* (aleurone color, dominant to *c*), and *c* (no aleurone color when homozygous). The interactions of the color factor bronze *(bz)* with *I* and *C* have been described by RHOADES (1952). Bronze phenotypes are visible in the endosperm only in the absence of *I* and presence of *C*. Endosperm composition is determined by genes *Sh* (*sh*, shrunken endosperm) and *Wx* (*wx*, waxy starch). Normal starch (*Wx*) stains blue with KI plus I_2 solution while waxy starch is either colorless or stains brown with iodine solution. At pachytene the genes *I*, *Sh*, and *Bz* are located close together in chromosome 9 their general position occupying a point slightly less than the distal third of the short arm. *Wx* is located close to the middle of the short arm. (MCCLINTOCK, personal communication). The crosses used in these experiments were either *c sh wx* × *C Sh Wx* or *C sh bz wx* × *I Sh Bz Wx*, permitting a study of three or four linked genes, respectively, in the short arm of chromosome 9. All F_1 kernels were scraped and stained in bulk with iodine solution, then examined microscopically for the appearance of nonstaining areas of waxy starch.

The use of chromosome 9 markers and the reading of mosaic patterns resulting from breakage-fusion-bridge (B.-F.-B.) cycles originated with the work of MCCLINTOCK (1951), who described the phenotypes expected from breaks occurring at various positions along the short arm of chromosome 9. For a comprehensive discussion of this subject see FABERGÉ (1956). In these experiments any deviations from the expected order of loss were placed in the "complex" class as the first point of chromosome breakage in these kernels is often impossible to determine.

The loss of dominant marker genes occurring spontaneously or induced by DEB were classified according to the number of genes involved and to the extent of the deficient area of the kernel. Fractional or mosaic patterns occupying less than one eighth of the kernel were excluded since they are both difficult to classify accurately, and their frequency is not increased appreciably by the DEB treatment (KREIZINGER 1958).

Preparation of DL-*diepoxybutane*

Since diepoxybutane may exist in several isomeric forms, the method of preparing and purifying the DL-compound used in these experiments is of importance. The stereospecific chemical used was substantially pure DL-diepoxybutane,

having been made from cis-2-butene-1,4-diol by bromination followed by dehydrobromination with dry potassium hydroxide. The material was carefully fractionated through a 30-inch Podbielniak column and the fraction, b.p. 58–60° (35mm), was collected. The DL-diepoxybutane was prepared under the direction of DR. D. D. PHILLIPS, formerly of Cornell University.

Methods of pollen treatment

Diepoxybutane was introduced into the pollen by one of two procedures. In the cut tassel method of treatment, tassels in the second or third day of pollen shedding were selected. Cut ends of the tassels were placed in flasks containing 200 ml of 0.2 percent (2.2×10^{-3}M) DEB solution. After 18 hours of treatment pollen was taken from the tassels, and pollinations were made immediately in the field. Pollen from a single tassel was used to pollinate one multiple recessive plant. The same procedure was followed for controls except that the solutions contained no DEB.

In the second method of treatment a wick was used to introduce DEB into the plant. Tassels just commencing to shed pollen were splinted below the tassel with a piece of wood to provide support. A hole five mm in diameter was bored into the stalk of the plant four inches below the lowest tassel branch, and a piece of woven glass wicking was pushed securely into the hole with forceps. The other end of the wick was placed in a one dram vial containing 4.5 cc of 0.2 percent DEB. The vial was attached to the stalk with masking tape, and an additional piece of tape was placed over the wick area to reduce evaporation. Although the vial was usually empty within two to four hours, it was not removed until the following day. The pollen was collected 20 hours after starting the treatment and at successive 24-hour periods for four days.

Statistical analysis

The t test and analysis of variance were used throughout for comparisons between treatment means. In the wick method of treatment the frequency of mutant kernels resulting from pollination with individual treated tassels differed significantly ($P = 0.05$) from tassel to tassel. Therefore, chi-square tests, which require that sampling within treatments be from a homogeneous population, are not applicable. In each case the analysis was made on the percent mutant kernels within individual ears.

Method used to calculate maps of chromosome 9 from induced breakage frequencies

The short arm of chromosome 9 was considered to have a length of 100 percent for determination of the relationship between frequency of breakage and the distance between marker genes along the arm. The mutant kernels from each ear of corn were tabulated according to the position of the original break between marked regions along the chromosome arm. Within an individual ear the number of breaks between each two marker genes was converted to a percentage of the total number of kernels showing chromosome breaks in that ear. Means (in

percent) were calculated for each chromosome region from all ears within each of the five days of pollination in the wick method of treatment, the two methods of treatment, and the two stocks used. These means were compared by appropriate t tests or analysis of variance. Chi-square tests are not valid because, as will be shown later, tassels treated with DEB differ from each other in the manner that breaks are apportioned along the chromosome arm and thus do not represent a homogeneous population.

RESULTS

The effectiveness of DEB as a chemical mutagen in maize pollen

The marked ability of DEB to induce losses of endosperm marker genes in maize is evident from Tables 1 and 2. The number of mutant kernels resulting from DEB treatment is either 50 or 60 times greater than the number observed in the control depending on the method of treatment. The statistical analysis shows that the difference between the frequency of mutant kernels in the DEB treated and control ears is significant at the $P = 0.05$ level (see Table 3). This difference was unaffected by the year of treatment, the stocks used, or any of the possible interactions between them (KREIZINGER 1958). For purposes of analysis it is assumed that the action of the mutagen is mainly in breaking chromosomes since all evidence, discussed below, indicates that this is by far the most important effect. The classification of kernels by B.-F.-B. cycles, interstitial losses, and terminal losses are shown for stock *I Sh Bz Wx* in Table 1 and for stock *C Sh Wx* in Table 2.

In the wick method of treatment a single tassel was used for five successive days of pollination. Only tassels in which all five days of pollination were represented by an ear were used in the statistical analyses. However, kernels from all ears resulting from the wick method are tabulated in Table 2. The means from the five successive days of pollination did not differ significantly in either the

TABLE 1

Type and frequency of mutant endosperms induced by 0.2 percent DEB treatment of maize pollen in the cross C sh bz wx × I Sh Bz Wx *using the cut tassel method of treatment*

		Classification of kernels													
		B.-F.-B. cycles starting in				Interstitial losses		Terminal losses				More complex			
Treatment	Year	*E-I*	*I-Sh*	*Sh-Bz*	*Bz-Wx*	*ShWx*	*Wx*	*I*	*Sh I*	*Bz Sh I*	*Wx Bz Sh I*		Total affected	Total examined	Percent affected
0.2%	1956	117	4	4	28	3	6	15	2	8	163	7	357	3314	10.8
	1957	44	1	1	10	.	1	3	1	2	57	3	123	920	13.4
	Total	161	5	5	38	3	7	18	3	10	220	10	480	4234	11.3
Control	1956	2	2	.	.	.	.	1	.	.	9	1	15	6377	0.2
	1957	1	.	.	.	.	.	.	.	.	.	.	1	874	0.1
	Total	3	2	.	.	.	.	1	.	.	9	1	16	7251	0.2

TABLE 2

Type and frequency of mutant endosperms induced by 0.2 percent DEB treatment of maize pollen in the cross c sh wx × C Sh Wx

			Classification of kernels											
			B.-F.-B. cycles starting in			Interstitial losses		Terminal losses			More complex			
Treatment	Method	Year	*E-C*	*C-Sh*	*Sh-Wx*	*ShWx*	*Wx*	*C*	*CSh*	*CShWx*		Total affected	Total examined	Percent affected
0.2%	Cut													
	tassel	1956	74	7	26	.	3	6	17	153	3	289	2436	11.8
		1957	44	0	23	1	1	4	3	85	1	162	1145	14.2
		Total	118	7	49	1	4	10	20	238	4	451	3581	12.6
	Wick	1957	313	21	97	0	17	26	37	652	9	1168	11578	10.1
	Grand total		431	28	146	1	21	36	57	890	13	1619	15159	10.7
Control	Cut													
	tassel	1956	5	.	1	.	.	1	.	2	.	9	4338	0.2
		1957	.	.	.	.	.	2	.	.	.	2	1548	0.1
	Wick	1957	5	1	.	.	.	5	.	2	.	13	5711	0.2
		Total	10	1	1	.	.	8	.	4	.	24	11597	0.2

TABLE 3

Statistical comparison of the frequency of mutant endosperms induced by DEB in c sh wx × C Sh Wx *and in* C sh bz wx × I Sh Bz Wx *using the cut tassel method of treatment*

	c sh wx × C Sh Wx				*C sh bz wx × I Sh Bz Wx*			
	1956		1957		1956		1957	
	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
No. ears	40	24	24	6	59	51	15	4
	percent				percent			
Total	670.0	5.2	342.8	0.8	780.3	13.0	193.8	0.4
Means	16.8	0.2	14.3	0.1	13.2	0.2	12.9	0.1
$S_{\bar{x}}$	1.8	0.1	1.4	0.1	1.2	0.1	1.5	0.1

treated or control material. Therefore, the five days of pollination were combined to determine the mutagenic effectiveness of DEB when introduced by the wick method. The unweighted five day DEB treatment mean of 10.6 percent is significantly different from the unweighted control mean of 0.4 percent at the $P = 0.01$ level providing further evidence of the ability of DEB to induce mutation in maize. The cut tassel method of treatment is significantly ($P = 0.05$) more effective than the wick method in the induction of marker losses. However, following the wick method of treatment, ears usually bear many more kernels which results in a much greater total number of mutant kernels for the number of pollinations made (KREIZINGER 1958).

Induced chromosome breaks related to marked regions in the short arm of chromosome 9

Before comparing the number of breaks between markers with the relative distances between them, it was necessary to determine what effect, if any, the two stocks, two years, and two methods of treatment had on the way breaks were distributed along the arm. This distribution is not influenced by the year of treatment or the interaction of years with stocks following the cut tassel method of treatment (KREIZINGER 1958). However, there is a surprising difference between the two stocks in the frequency of breakage induced within the region between *I (C)* and the end of the short arm and between *Sh* and *Wx*. The amount of breakage distal to *I* in stock *I Sh Bz Wx* is about ten percent higher than in stock *C Sh Wx* (see Table 4)—a difference which is significant at the P = 0.05 level.

TABLE 4

The relative frequency of breakage within marked regions of chromosome 9 in stocks C Sh Wx *and* I Sh Bz Wx

Region between markers	Mean of stock *C Sh Wx* percent	Mean of stock *I Sh Bz Wx* percent	Mean difference
End–*C (I)*	26.0	35.7	9.7* ± 4.6
C –*Sh*	3.8	4.4	0.6 ± 1.7
Sh –*Wx*	22.2	13.9	8.3 ± 4.2
Wx–Centromere	48.0	46.0	2.0 ± 4.3

* Significant at the P = 0.05 level.

The difference in breakage frequency distal to *I* in the two stocks is almost entirely compensated for between *Sh* and *Wx* where the frequency of breaks in stock *C Sh Wx* is eight percent higher than in stock *I Sh Bz Wx*. These latter figures are highly suggestive of a real difference although the t value is not quite significant.

The *C Sh Wx* stock was used exclusively in the wick method of treatment. With the exception of the region between *Sh* and *Wx*, the frequency of breaks within the marked regions of the short arm of chromosome 9 did not differ significantly among any of the five days of pollination. However, in the region between *Sh* and *Wx* there was a significant difference (P = 0.05) among successive days of pollination as well as a highly significant difference (P = 0.01) among tassels in the frequency of breakage within this region (KREIZINGER 1958). Since all of the difference among days could be attributed to the fifth day of pollination, data from the fifth day were omitted from further comparisons. The analysis was based on DUNNETT's method for comparing treatment means with a control, in this case using the first day of pollination as the control (DUNNETT 1955). There was no difference between the two methods of treatment on the apportionment of breaks along the arm.

After finding that the distribution of breaks along the chromosome arm was

not influenced by the years or methods of treatment, it was possible to relate the frequency of breaks between marked loci to the actual relative distances between them in each stock. As shown in Figure 1 the distances between loci as determined cytologically and by breakage frequencies are remarkably similar. The percent of breakage between loci in the *C Sh Wx* stock compares almost exactly with the frequency expected if breakage and restitution are at random along the chromosome arm. However, in the *I Sh Bz Wx* stock there appears to be a relative excess of breaks distal to *I* and a dearth of these events between *Sh* and *Wx*. This may indicate some type of differential breakage or restitution in the *I Sh Bz Wx* stock.

The relationship between sectoring and whole kernel effects following DEB treatment of maize pollen

Sectoring, in which only a portion of the kernel shows the loss of the dominant marker genes, is observed very frequently following DEB treatment of maize pollen. The loss sectors in the kernels were classified roughly according to size as one eighth, one fourth, one half, three fourths, or entire. Kernels with B.-F.-B. cycles initiated distal to *C* or *I* were not classified by sector size. Two points were revealed by the classification: (1) The frequency of sectorial to entire kernel losses of marker genes was approximately two to one, and this frequency was not influenced by the method or year of treatment or the stock used. (2) The size of the sectors formed a nearly normal frequency distribution about the mean value of one half of the endosperm (KREIZINGER 1958). The possible causes of sectoring will be considered below.

DISCUSSION

The high frequency of mutant kernels obtained following DEB treatment of the pollen places this chemical with X-rays and nitrogen mustards as powerful inducers of mutant endosperms in maize. Although no direct comparisons among these mutagens have been made, the frequency of mutation resulting from DEB treatment of maize pollen is very similar to that obtained by FABERGÉ (1956) using 1500r of X-rays on pollen from an *I Sh Bz Wx* stock. The frequency of

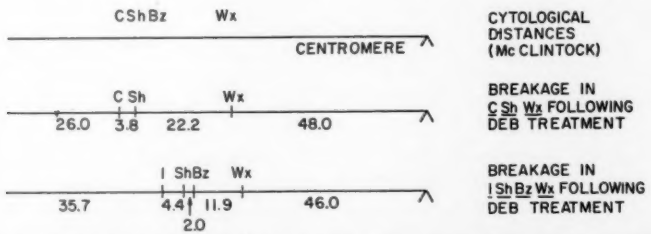

FIGURE 1.—Distances between marked loci in the short arm of chromosome 9 as determined cytologically at pachytene and as determined from breakage frequencies (in percent) following DEB treatment.

endosperm mutations induced by DEB is also similar to the 12 percent obtained by GIBSON *et al.* (1950) using moderately severe doses of nitrogen mustards.

Differential sensitivity of maize pollen to DEB introduced during each of the five days prior to pollen shedding was also investigated. It has been reported by SINGLETON and CASPER (1954) that the time of greatest sensitivity for mutation production in maize pollen treated with gamma irradiation is a few days before the pollen is shed. The mutation rate drops sharply for irradiations applied during the last two days before pollen shedding. In the radiation treatments of SINGLETON and CASPER maize tassels were irradiated in a timed sequence before pollen shedding. In the DEB wick method, tassels were treated at one time, and pollen was collected on successive days following treatment. While the number of days elapsing between the treatment of any individual pollen grain with DEB and its time of shedding undoubtedly varied because of environmental factors, there was no indication of increased sensitivity with earlier treatment as reported for gamma irradiation. In so far as the two groups of data can be compared directly, the results indicate some fundamental difference between the action of gamma irradiation and DEB on the production of mutations in maize pollen. Further study employing both mutagens under the same conditions are in progress.

There is no evidence that gene mutation is markedly increased by DEB treatment of maize pollen. The total number of single gene losses of *I*, *C*, and *Wx* observed was only 82 out of 22,410 kernels. This is a lower frequency of single losses of *I (C)* and *Wx* than the 43 observed by FABERGÉ (1956) in 6,782 kernels following X-ray treatment of the pollen although the over-all mutation rate from the two types of treatment was similar. The studies of STADLER (1954) and NUFFER (1957) have shown that all single gene losses observed following X-ray treatment of maize pollen are chromosomal deletions, not true point mutations. Therefore, since the number of single gene losses after DEB treatment was proportionately fewer than following X-ray treatment, it can be tentatively inferred that the single gene losses following DEB treatment were largely chromosomal deficiencies. A few spontaneous gene mutations would be expected in a sample of over 22,000 kernels; these would be included among the 82 single gene losses.

The paucity of gene mutations following DEB treatment is in agreement with the suggestion that maize reacts differently to mutagenic agents than does Drosophila or Neurospora. Although X-rays are known to cause true gene mutations in both these organisms, they are induced extremely rarely, if at all, in maize. Similarly, gene mutation in maize is not markedly increased by DEB treatment although in Neurospora this type of mutation is greatly increased by the same chemical (KÖLMARK and GILES 1953).

From the use of linked genes in the short arm of chromosome 9 it was possible to determine whether breakage and restitution were at random along this arm following DEB treatment. A study of the effect of year, stock, and method of treatment on the position of breaks revealed that the two stocks differed sig-

nificantly ($P = 0.05$) in the amount of breakage distal to *I (C)*. The difference in the frequency of breakage between *Sh* and *Wx* in the two stocks also approached significance. There are three possibilities to account for these differences. (1) A marked region in one stock is more sensitive to breakage or less likely to restitute than the same region in the other stock. (2) The short arm of chromosome 9 differs in length in the two stocks either throughout the chromosome cycle or at the time of treatment. (3) The differences are due to sampling error.

The most plausible explanation for the discrepancy in the two stocks appears to be some type of differential breakage or restitution within one or both stocks. Credence for the explanation of genic control of breakage comes from three sources. (1) The studies of McCLINTOCK (1951) have established that spontaneous breakage in maize can be under genic control. However, it should be emphasized that in the present experiments there was no evidence of a Dissociator-Activator system having been induced by the treatment. (2) Crossing over appears to be under genic control in maize since stocks which differ in crossover frequency between marked loci have these loci in the same cytological position at pachytene (McCLINTOCK, personal communication). According to the recent work of TAYLOR (1957) chromosome breakage is a prerequisite to crossing over. Therefore, since crossing over is governed by the genotype, the chromosome breaks involved must be similarly controlled. (3) The work of LINNERT (1951) indicates that induced breaks are also genicly controlled. Treatment of pachytene chromosomes of maize with urethane plus KCl resulted in the breaking of both homologues of a pair at the same region more often than would be expected on the basis of chance.

Differences in the length of the short arm of chromosome 9 in the two stocks at time of treatment cannot be responsible for the differences between stocks. If the region distal to *I* in stock *I Sh Bz Wx* were actually longer than the same region in stock *C Sh Wx*, all other regions in stock *I Sh Bz Wx* should show correspondingly fewer breaks rather than only the region between *Sh* and *Wx*.

The possibility that the differences between stocks are due simply to sampling error cannot be excluded. However, the excellent agreement in frequency of breakage within identical regions of the chromosome arm in the same stock under different years and methods of treatment and the wide divergence between stocks regardless of years, suggest that this is a real difference.

The relative distances between marked loci based on the number of breaks occurring between them were compared with the positions of the markers as observed at pachytene. The close agreement in the position of loci as determined by these two methods is of considerable interest since it suggests that breakage induced by DEB is approximately at random along the chromosome arm.

The relative location of breaks induced by DEB treatment within chromosome 9 were also compared with those induced by X-rays using the data of FABERGÉ (1956) in which maize pollen from an *I Sh Bz Wx* stock was treated with 1500r of X-rays and was used to pollinate a *C sh bz wx* stock. Variations in the breakage frequencies within identical regions following DEB or X-ray treatment are prob-

ably no greater than can be expected on the basis of heterogeneity in the material and differences in experimental procedure.

One factor not considered in the DEB data is the conclusion of FABERGÉ (1956) that stable, terminal deletions occur only rarely, if at all, in maize endosperm following ultraviolet or X-ray treatment of the pollen. If stable end losses do not occur, a loss not followed by fusion of the two sister chromatids must be considered to involve two breaks, one of which would not be detected if occurring distal to *I*. The fact that functionally stable chromosome ends are produced in maize following radiation by alpha particles (FABERGÉ 1959) indicates that the behavior of the chromosome is dependent on the type of treatment. There is no *a priori* reason to expect the production of stable, terminal ends to be the same following DEB treatment as following either type of radiation. Computations to determine whether stable, terminal deletions are produced following DEB treatment are not feasible since data were not from a homogeneous population and cannot be pooled.

The frequency of sectoring following DEB treatment of maize pollen was about two fractional losses to one entire loss, a ratio similar to that found following ultraviolet or nitrogen mustard treatment (STADLER and SPRAGUE 1936; GIBSON, BRINK, and STAHMANN 1950). By contrast, sectoring is relatively infrequent in maize following X-ray treatment (STADLER and SPRAGUE 1936; NUFFER 1957).

The hypotheses which have been suggested to explain the frequent occurrence of sectoring in maize and Drosophila following ultraviolet or chemical treatment are: (1) a multiple strand nature of the chromosome leading to the possibility that the treatment affects only a portion of the subunits rather than the entire chromosome, (2) interference with chromosome duplication resulting in subsequent chromosome or chromatid breakage, (3) induction of an unstable genic state, and (4) influence of the treatment on the chromosome matrix.

It has frequently been suggested that ultraviolet and chemicals break chromatids or smaller chromosome subunits as opposed to the action of X-rays which break whole chromosomes. Sectors smaller or larger than one half are envisioned as the result of the sorting out of subunits which were affected at the time of treatment. However, MULLER (1940) observed that if the entire chromosome is broken but held together until time of division, one of the resulting broken chromatids may restitute while the other heals. The loss of only one acentric fragment bearing dominant genes would result in a sector of recessive tissue.

It has also been suggested that ultraviolet and chemicals interfere with chromosome duplication rather than actually breaking chromosomes. Chemical interference with duplication is supported by the fact that epoxides react *in vitro* with both phosphate groups and amino acids (Ross 1958; STACEY, COBB, COUSINS, and ALEXANDER 1958).

The hypothesis of genic instability resulting in point mutation as proposed by AUERBACH (1951) for mosaicism in Drosophila does not seem a satisfactory answer for sectoring in maize. As previously discussed, maize responds differently to X-ray and chemical treatment than does Drosophila in that gene mutation is

very rare in maize following these treatments. Therefore, genic instability does not seem a satisfactory explanation for sectoring in *Zea mays*.

The fourth hypothesis for sectoring involves changes in the chromosome matrix, a suggestion which has been proposed by SWANSON (1944) to explain the anomalous results obtained from combined ultraviolet and X-ray treatments in Tradescantia. Diepoxybutane may conceivably exert an effect on the chromosome matrix which renders it more difficult for chromatids or acentric fragments to separate. Such an effect would increase the occurrence of restitution and of sectoring when the entire chromosome is broken by the treatment.

SUMMARY

The mutagenic effects of diepoxybutane treatment of maize pollen were studied. Losses of dominant marker genes in the endosperm of the resulting kernels were used to evaluate the mutagenicity of the diepoxybutane. One stock contained three, the other four marker genes located on the short arm of chromosome 9.

Diepoxybutane was found to be a powerful inducer of mutant endosperm sectors in maize. The proportion of F_1 kernels showing single or multiple gene losses was about 12 percent. There was no evidence that the frequency of gene mutation was markedly increased by diepoxybutane treatment although some single gene losses may have been "point" mutations.

The distance between marked loci in the short arm of chromosome 9 as determined by the relative frequency of breaks occurring between these loci were very similar to the cytological distances as determined at pachytene. This was considered evidence that breakage resulting from diepoxybutane treatment was induced approximately at random along the chromosome arm. However, the two stocks used differed significantly in the number of breaks observed distal to *I*. It is suggested that this may be due either to differential sensitivity of some chromosomal regions to breakage or to sampling error.

About twice as many kernels had sectors of mutant tissue as had exclusively mutant tissue in the endosperm. The size of mutant sectors formed a nearly normal distribution about the most frequent values of one half the endosperm. The possible causes of sectoring are considered.

ACKNOWLEDGMENTS

The author wishes to express her deepest appreciation to DR. HAROLD H. SMITH for his guidance and interest throughout the course of this research. To DR. DOUGLAS ROBSON, she is indebted for assistance with the statistical analysis. Special thanks are expressed to DRS. M. EMMERLING, H. SMITH, and D. STEFFENSON for helpful criticisms of the manuscript.

LITERATURE CITED

AUERBACH, C., 1951 Induction of changes in genes and chromosomes. Cold Spring Harbor Symposia Quant. Biol. **16**: 199–213.

BIRD, M. J., and O. G. FAHMY, 1953 Cytogenetic analysis of the action of carcinogens and tumor inhibitors in *Drosophila melanogaster*. I. 1:2,3:4-diepoxybutane. Proc. Roy. Soc. Lond. B **141**: 556–578.

DEMEREC, M., H. MOSHER, J. HEMMERLY, I. BLOMSTRAND, Z. E. DEMEREC, P. L. FITZGERALD, S. W. GLOVER, J. E. HANSON, F. J. NIELSEN, and T. YURA, 1954 Bacterial genetics—I. Carnegie Inst. Wash. Ybk. **53**: 225–241.

DUNNETT, C. W., 1955 A multiple comparison procedure for comparing several treatments with a control. J. Am. Stat. Assoc. **50**: 1096–1121.

FABERGÉ, A. C., 1955 Types of chromosome aberrations induced by ethylene oxide in maize. Genetics **40**: 571.

1956 The analysis of induced chromosome aberrations by maize endosperm phenotypes. Z. Ind. Abst. Vererb. **87**: 392–420.

1959 Production by alpha particles of functionally stable broken chromosome ends in maize. Genetics **44**: 279–285.

FAHMY, O. G., and M. J. BIRD, 1953 Chromosome breaks among recessive lethals induced by chemical mutagens in *Drosophila melanogaster*. Heredity (Suppl.) **6**: 149–159.

FAHMY, O. G., and M. J. FAHMY, 1956 Cytogenetic analysis of the action of carcinogens and tumor inhibitors in *Drosophila melanogaster*. J. Genet. **54**: 146–164.

GIBSON, P. B., R. A. BRINK, and M. A. STAHMANN, 1950 The mutagenic action of mustard gas on *Zea mays*. J. Heredity **41**: 232–238.

KÖLMARK, G., 1953 Differential response to mutagens as studied by the Neurospora reverse mutation test. Hereditas **39**: 270–276.

KÖLMARK, G., and N. H. GILES, 1953 Studies on chemical mutagens using the Neurospora back mutation test. Rec. Genet. Soc. Am. **22**: 66.

KÖLMARK, G., and M. WESTERGAARD, 1953 Further studies on chemically induced reversions at the adenine locus of Neurospora. Hereditas **39**: 209–224.

KREIZINGER, J. D., 1958 Diepoxybutane as a chemical mutagen in maize. Ph.D. Thesis. Cornell University. 139 pp.

LINNERT, G., 1951 Die Einwirkung von Chemikalien auf die Meiosis. Z. Ind. Abst. Vererb. **83**: 422–428.

LOVELESS, A., 1951 Qualitative aspects of the chemistry and biology of radiomimetic (mutagenic) substances. Nature **167**: 338–342.

MCCLINTOCK, B., 1951 Chromosome organization and genic expression. Cold Spring Harbor Symposia Quant. Biol. **16**: 13–47.

MULLER, H. J., 1940 An analysis of the process of structural change in chromosomes of Drosophila. J. Genet. **40**: 1–66.

NUFFER, M. G., 1957 Additional evidence on the effect of X-ray and ultraviolet radiation on mutation in maize. Genetics **42**: 273–282.

RHOADES, M. M., 1952 The effect of the bronze locus on anthocyanin formation in maize. Am. Naturalist **86**: 105–108.

ROSS, W. C. J., 1958 *In vitro* reactions of alkylating agents. Annals N.Y. Acad. Sci. **68**: 669–681.

SINGLETON, W. R., and A. L. CASPER, 1954 Effect of time of gamma radiation on microspore mutation rate in maize. Rec. Genet. Soc. Am. **23**: 66.

STACEY, K. A., M. COBB, S. COUSINS, and P. ALEXANDER, 1958 The reactions of the "radiomimetic" alkylating agents with macro-molecules *in vitro*. Annals N.Y. Acad. Sci. **68**: 682–701.

STADLER, L. J., 1954 The gene. Science **120**: 811–819.

STADLER, L. J., and G. F. SPRAGUE, 1936 Genetic effects of ultraviolet radiation in maize. I. Unfiltered radiation. Proc. Natl. Acad. Sci. U.S. **22**: 572–578.

SWANSON, C. P., 1944 X-ray and ultraviolet studies on pollen tube chromosomes. I. The effect of ultraviolet (2537Å) on X-ray induced chromosomal aberrations. Genetics **29**: 61–68.

TAYLOR, J. H., 1957 The time and mode of duplication of chromosomes. Am. Naturalist **91**: 209–221.

UMALI, D. L., 1949 Induction of mutations in maize with nitrogen mustards. Ph.D. Thesis. Cornell University. 103 pp.

WESTERGAARD, M., 1957 Chemical mutagenesis in relation to the concept of the gene. Experientia **13**: 217–224.

THE THEORY AND ANALYSIS OF DIALLEL CROSSES. III

B. I. HAYMAN

Applied Mathematics Laboratory, D.S.I.R., Private Bag, Christchurch, New Zealand

Received June 23, 1959

THE theory and methods of analysis of the diallel cross, as developed over the last ten years, have reached the stage where three main lines of approach are evident—those expounded by GRIFFING, by HAYMAN and JINKS and by KEMPTHORNE. Though these exponents will be aware of the relationship between their own lines of approach and the other two, the plant or animal breeder may have difficulty in choosing an experimental design and a method of analysis from amongst the various suggestions that have been published. These suggestions have actually been advanced to meet different needs and circumstances, although this has not always been made clear, and the breeder who does not realize this may feel forced to base his choice on adherence to one of what he thinks are rival methods rather than on his experimental needs. In this paper an attempt has been made to give the various aims of diallel cross experiments, to indicate which designs and methods meet these aims and to interrelate and extend the various methods. Since GRIFFING has related some of his work to KEMPTHORNE's and since some misconceptions have arisen about the methods of HAYMAN and JINKS, the emphasis is on the work of the last named.

General survey

A diallel cross is taken to be a set of inbred lines together with the complete set of their F_1 progeny—a total of n^2 families. Later generations are sometimes included. A diallel table is a set of n^2 observations derived from a diallel cross.

Diallel analyses differ in three main ways—in the material ultimately under investigation, in the postulated underlying genetic mechanism and in the methods of estimation. The interest of some breeders lies in a particular set of inbred lines but others are interested in a population of which these lines could be considered a sample. This is the main line of division between the methods of KEMPTHORNE and the methods of HAYMAN and JINKS. HAYMAN (1954a, 1957, 1958a) and JINKS (1954, 1956) studied the genetics of the differences within a set of inbred lines. KEMPTHORNE (1956) expressed some of the formulae of HAYMAN and JINKS in terms of the parameters of the random mating population from which the inbred lines might have been derived by inbreeding. GRIFFING (1956a) pointed out that both of these approaches were possible and he presented some examples comparing them.

Basically the approach of HAYMAN and JINKS was the estimation of selected parental and progeny means and the testing of differences between them. They also expressed the variation amongst the means in terms of variance components that were descriptive strictly only of a population from which the selected lines

might have been sampled. Nevertheless, these components give an approximate description of the genetic situation in a given set of inbred lines. Their analysis lay in the domain of EISENHART's (1947) Model I, while KEMPTHORNE's lay in the domain of Model II, but they also, to paraphrase EISENHART, "looked at their Model I analysis through Model II spectacles".

The second difference is between what may be roughly termed the statistical and the genetical models of gene action. Here the contrast lies between GRIFFING on the one hand and HAYMAN, JINKS and KEMPTHORNE on the other. HAYMAN and JINKS worked in terms of simple but reasonably general multiple gene models and HAYMAN's (1954a) five components of genetic variation, D, F, H_1, H_2 and h^2 together with the (V_r, W_r) graph approximately represent the genetic situation in a given set of inbred lines. GRIFFING (1956a,b, 1958) emphasizes the statistical concepts of general and specific combining ability, with a loss of genetical information, since he generally neglects the inbred lines themselves. KEMPTHORNE (1956) and MATZINGER and KEMPTHORNE (1956) also describe the underlying genetic model.

The third difference, which is allied to the second, is that between estimation from analysis of variance and more general estimation procedures. Here GRIFFING and KEMPTHORNE differ from HAYMAN and JINKS. HAYMAN (1954a) showed that genetic variation in a given diallel cross could in many cases be divided approximately into five components D, F, H_1, H_2 and h^2 and gave methods of estimating these from statistics involving both parents and offspring. Some of these statistics were additional to those supplied by the analysis of variance of the diallel table as HAYMAN (1954b) pointed out. KEMPTHORNE (1956) and GRIFFING (1958) described five components of variation for which KEMPTHORNE suggested the need of estimation procedures. The genetic components of HAYMAN and JINKS are interpretations of these components in terms of their particular genetic model. Both KEMPTHORNE and GRIFFING realized that the two genetical mean squares from their analyses of variance of the diallel table would not supply estimates of five components. GRIFFING (1956b, 1958) restricted his analysis to the F_1 families; their genetic variation could be expressed in terms of two statistical parameters, the variances of general and specific combining abilities. This, of course, also restricted the genetic situations that could be discussed to those characterized by only two components of variation.

Accompanying the estimation problem is the problem of tests of significance about which there is more agreement. Exact significance tests have been of two kinds. The first is the analysis of variance of the diallel table, given originally by YATES (1947). HAYMAN (1954a) pointed out that it provided a test for additive genetic variation in the absence of dominant and epistatic variation or a test of dominance variation in the absence of epistatic variation and KEMPTHORNE (1956) confirmed these results. GRIFFING (1956a,b) showed that the analysis supplied simultaneous tests of both general and specific combining ability. GRIFFING's simultaneous tests seem superior to HAYMAN's hierarchical tests, but in fact they are equivalent because, in the presence of specific combining ability,

general combining ability is a compound of dominance, or epistasis, or both, with additive genetic effects. The second kind of exact test involves other generations and is based on MATHER's (1949) so-called linear scaling tests. The extension of one of these to the diallel cross including parental, F_1 and F_2 families was given by HAYMAN in 1957. A useful approximate test of significance of components of variation uses empirical standard errors from replication (NELDER 1953). HAYMAN (1954a, 1958a) also obtained standard errors from least squares solutions for components and GRIFFING (1956a) also used theoretical errors.

We can sum up as follows. The methods of HAYMAN and JINKS, founded on those of MATHER (1949) supply a great deal of information about a set of inbred lines and its offspring. The combination of the analysis of variance of the diallel table with the analysis of variance of W_r-V_r or the test involving F_2 families sets the level of complexity of the genetic situation at solely additive, additive and dominant, or additive, dominant and epistatic. Except in the last and most complicated circumstance, the (V_r,W_r) graph and the genetic components supply a detailed description of the relative genetic properties of the inbred lines. Even in the presence of epistasis the F_1 (V_r,W_r) graph or consideration of further generations provides a great deal of information about the epistasis. GRIFFING (1956a) has shown how to estimate the general and specific combining abilities of such a set of lines.

When the inbred lines are considered to be a sample of the lines resulting from inbreeding a random-mating population, GRIFFING's (1956a,b) analyses of variance supply tests and estimates for the variances of general and specific combining abilities in the sampled population.

We will devote the remainder of this paper to extending the approximate analysis of fixed inbred lines of HAYMAN and JINKS to the exact analysis of inbred lines considered as a sample from an inbred but originally random-mating population. Their notation will then cover all cases of importance to breeders. To do so we will in effect reinterpret KEMPTHORNE's (1956) model of the diallel table, but we will show how all five components of the variation and their standard errors can be estimated.

Statistical components of variation

From a statistical sampling point of view we may regard an $n \times n$ diallel table as a single observation of a set of n^2 variables in a multivariate distribution. With the genetical background to the table in mind we assume that reciprocal variables are identical and group the large number of parameters that characterize such a distribution into six classes, each with a common value. These values are

μ_0 = mean of a diagonal variable,

μ_1 = mean of a nondiagonal variable,

σ_0^2 = variance of a diagonal variable,

c_{01} = covariance between a diagonal variable and any other variable in the same array (row or column of the table),

σ_1^2 = variance of a nondiagonal variable,

c_{11} = covariance between nondiagonal variables in the same array. Other

covariances are assumed to be zero. The subscripts, 0 and 1, denote the two generations, parental and F_1, under observation.

The expected variation in the diallel table is completely described by these variances and covariances together with

$$\mu = \mu_1 - \mu_0$$

These parameters are related to those of KEMPTHORNE (1956) and GRIFFING (1958) as follows

HAYMAN	KEMPTHORNE	GRIFFING
μ_0	μ_I	μ_I
μ_1	μ	μ
σ_0^2	σ_I^2	σ_I^2
c_{01}	C	σ_{gI}
σ_1^2	σ_G^2	$2\sigma_{gca}^2 + \sigma_{sca}^2$
c_{11}	$cov(P.O)$	σ_{gca}^2

We describe two methods of estimating these parameters, firstly from the expectations of statistics and secondly by maximum likelihood. Let x_{ij} ($i,j = 1,2, - - - n$ and $x_{ij} = x_{ji}$) be the $\frac{1}{2}n(n+1)$ variables of the diallel table of family and reciprocal means. Define

$x = \Sigma x_{ii}$ (summed over all i), the sum of diagonal terms,
$X_i = \Sigma x_{ij}$ (all $j \neq i$), the sum of the ith array excluding its diagonal term,
$X = \Sigma x_{ij}$ (all i and j, $i \neq j$)
$= \Sigma X_i$ (all i), the sum of the $n(n-1)$ nondiagonal terms. Summation over i and j is not restricted to $i < j$. This convention eliminates an awkward factor of 2 from many formulae.

We define also the second degree statistics

$$nS_0 = n\Sigma x_{ii}^2 - x^2$$
$$nS_{01} = n\Sigma x_{ii}X_i - xX$$
$$n(n-2)S_{11} = n\Sigma X_i^2 - X^2$$
$$2(n-1)(n-2)S_1 = (n-1)(n-2)\Sigma x_{ij}^2 - 2(n-1)\Sigma X_i^2 + X^2$$

The two sums of squares, S_{11} and S_1, are those in GRIFFING's (1956a,b) analysis of variance of nondiagonal terms. The subscript of each statistic is generally the same as the subscript of the major component of its expectation.

If E_0 and E_1 are the error variances of diagonal and nondiagonal means the expectations of the statistics are

$$\epsilon x = n\mu_0$$
$$\epsilon X = n(n-1)\mu_1$$
$$\epsilon S_0 = (n-1)(E_1 + \sigma_0^2)$$
$$\epsilon S_{01} = (n-1)(n-2)c_{01}$$
$$\epsilon S_{11} = (n-1)[E_1 + \sigma_1^2 + (n-4)c_{11}]$$
$$\epsilon S_1 = \tfrac{1}{2}n(n-3)(E_1 + \sigma_1^2 - 2c_{11})$$

Unbiassed estimators of the six parameters are then

$$\hat{\mu}_0 = x/n$$
$$\hat{\mu}_1 = X/n(n-1)$$
$$\hat{\sigma}_0^2 = S_c/(n-1) - \hat{E}_0$$
$$\hat{c}_{01} = S_{01}/(n-1)(n-2)$$
$$\hat{\sigma}_1^2 = 2[n(n-3)S_{11} + (n-1)(n-4)S_1]/n(n-1)(n-2)(n-3) - \hat{E}_1$$
$$\hat{c}_{11} = [n(n-3)S_{11} - 2(n-1)S_1]/n(n-1)(n-2)(n-3)$$

E_0 and E_1 are estimated from replicate diallel crosses or duplicate families and have been kept separate because inbred and crossbred individuals commonly have different error variances. Also of interest later is

$$\hat{\sigma}_1^2 - 2\hat{c}_{11} = 2S_1/n(n-3) - \hat{E}_1$$
$$= \hat{\partial}_2^2, \text{ say.}$$

In the genetic case this is the component of variation in specific combining ability.

The expected variances and covariances of the estimators of the means are

$$var\hat{\mu}_0 = (E_0 + \sigma_0^2)/n$$
$$cov(\hat{\mu}_0, \hat{\mu}_1) = 2c_{01}/n$$
$$var\hat{\mu}_1 = 2[E_1 + \sigma_1^2 + 2(n-2)c_{11}]/n(n-1)$$

The expected variances and covariances of the estimators of the statistical components of variation are in Table 1. n_0 and n_1 are the degrees of freedom of E_0 and E_1.

We discuss the properties of these estimators immediately and leave the more difficult derivation of the maximum likelihood estimators until later. The latter, though more complicated than, are not essentially different from, the unbiassed estimators.

The diagonal terms of Table 2 are the ratios, to the first order in n, of the square of each parameter to its sampling variance or, in other words, the approximate chi-square with one degree of freedom for testing the significance of the estimators of each parameter. For significance at 5, 1 or 0.1 percent these ratios should be about 4, 6½ or 11 respectively. Evidently σ_2^2 is estimated most accurately and a moderate sized diallel cross suffices for this parameter; when n_1 is very large the exact value of the chi-square is $\frac{1}{4}n(n-3)$, showing that at least six parents are necessary for significance at five percent. For σ_0^2, $\chi^2 = \frac{1}{2}(n-1)$ exactly when n_0 is very large, so that at least nine parents are necessary for the same accuracy of estimation. The situation is similar for the parameter c_{11} except that the accuracy of the tabulated approximation depends, to the next order in n, on the relative magnitudes of the parameters as well as on the magnitude of n. The accuracy of estimation of the other two parameters, σ_1^2 and c_{01}, depends directly on the relative magnitudes of the parameters. If c_{01} is small compared with σ_0^2 and c_{11} it cannot be estimated accurately in most experiments. On the other hand, the smaller size of c_{11} compared with σ_1^2 means that σ_1^2 is usually accurately estimated even in small diallel crosses.

It seems that a diallel cross containing ten parents would supply useful estimates of the parameters as long as the estimates of E_0 and E_1 are relatively ac-

TABLE 1

Variance matrix of unbiassed estimators of statistical components

	$\hat{\sigma}_0^2$	$\hat{c}_{01}$	$\hat{\sigma}_1^2$	$\hat{c}_{11}$	$\hat{\sigma}_2^2$
$\hat{\sigma}_0^2$	$\frac{2v_0^2}{n-1}+\frac{2E_0^2}{n_0}$	$\frac{2v_0c_{01}}{n-1}$	$\frac{4c_{01}^2}{n-1}$	$\frac{2c_{01}^2}{n-1}$	0
$\hat{c}_{01}$		$\frac{v_0v_3+(n-2)c_{01}^2}{(n-1)(n-2)}$	$\frac{4c_{01}v_3}{(n-1)(n-2)}$	$\frac{2c_{01}v_3}{(n-1)(n-2)}$	0
$\hat{\sigma}_1^2$			$\frac{8n(n-3)v_3^2+4(n-1)(n-4)^2v_2^2}{n(n-1)(n-2)^2(n-3)}+\frac{2E_1^2}{n_1}$	$\frac{4n(n-3)v_3^2-4(n-1)(n-4)v_2^2}{n(n-1)(n-2)^2(n-3)}$	$\frac{4(n-4)v_2^2}{n(n-2)(n-3)}+\frac{2E_1^2}{n_1}$
$\hat{c}_{11}$				$\frac{2n(n-3)v_3^2+4(n-1)v_2^2}{n(n-1)(n-2)^2(n-3)}$	$\frac{-4v_2^2}{n(n-2)(n-3)}$
$\hat{\sigma}_2^2$					$\frac{4v_2^2}{n(n-3)}+\frac{2E_1^2}{n_1}$

where $v_0=E_0+\sigma_0^2$; $v_1=E_1+\sigma_1^2$; $v_2=E_1+\sigma_1^2-2c_{11}$; $v_3=E_1+\sigma_1^2+(n-4)c_{11}$

TABLE 2

Diagonal: approximate chi-square test of significance of estimates of parameters. Off-diagonal: square of approximate correlations between estimators of parameters when error variances are neglected

	$\hat{\sigma}^2_0$	$\hat{c}_{01}$	$\hat{\sigma}^2_1$	$\hat{c}_{11}$	$\hat{\sigma}^2_2$
$\hat{\sigma}^2_0$	$\left[\frac{2}{n}+\frac{2E_0^2}{n_0 v_0^2}\right]^{-1}$	$\frac{2r^2}{1+r^2}$	r^4	r^4	0
$\hat{c}_{01}$		$\frac{nc_{01}^2}{v_0 c_{11}+c_{01}^2}$	$\frac{2r^2}{1+r^2}$	$\frac{2r^2}{1+r^2}$	0
$\hat{\sigma}^2_1$			$\left[\frac{8c_{11}^2}{nv_1^2}+\frac{2E_1^2}{n_1 v_1^2}\right]^{-1}$	$1-\frac{\sigma_2^4}{2nc_{11}^2}$	$\frac{\sigma_2^4}{2nc_{11}^2}$
$\hat{c}_{11}$				$\frac{n}{2}$	$\frac{2\sigma_2^4}{n^2c_{11}^2}$
$\hat{\sigma}^2_2$					$\left[\frac{4}{n^2}+\frac{2E_1^2}{n_1 v_1^2}\right]^{-1}$

where $r^2 = c_{01}^2/\sigma_0^2 c_{11}$; $v_0 = E_0 + \sigma_0^2$; $v_1 = E_1 + \sigma_1^2$

curate and that a smaller diallel cross would have little utility. This is to be expected for we are concerned here with estimating parameters in a hypothetical population of which any given diallel cross is but a sample.

The only circumstance in which a small diallel cross (less than ten parents) is of practical use is when the parents and crosses comprise the whole population under investigation. In this case the parameters to be estimated are those of this restricted group of parents and crosses alone, their accuracy being determined by replication and control of the environment and not by the sampling theory above. As we stated in the general survey this is the situation with which HAYMAN and JINKS, and to some extent GRIFFING, have been concerned.

Table 2 also contains, off the diagonal, the squares of the correlations between the estimators of the parameters, in most cases given to the first order in n. The error variances have been neglected here for simplicity; their inclusion reduces all correlations except that between the estimators of σ_1^2 and σ_2^2. The correlations of $\hat{\sigma}_0^2$ and $\hat{c}_{01}$ depend on the magnitude of c_{01} relative to σ_0^2 and c_{11}, measured by $r^2 = c_{01}^2/\sigma_0^2 c_{11}$. When c_{01} is relatively large the estimators of σ_0^2 and c_{01} are highly correlated with each other and with the estimators of σ_1^2 and c_{11} but when c_{01} is relatively small $\hat{\sigma}_0^2$ and $\hat{c}_{01}$ are nearly independent of each other and of $\hat{\sigma}_1^2$ and $\hat{c}_{11}$. The remaining correlation, that between $\hat{\sigma}_1^2$ and $\hat{c}_{11}$ behaves quite differently and indeed tends to unity in large diallel crosses. A relatively small value of the cor-

relation c_{11} ($<<n^{-3/2}\sigma_2^2$) does lead to a small correlation ($8n^{-3}$) between $\hat{\sigma}_1^2$ and $\hat{c}_{11}$, but such a small value of c_{11} is unlikely in genetical cases.

The estimator of the derived parameter, $\sigma_2^2 = \sigma_1^2 - 2c_{11}$, occupies a unique position in Table 2. As well as being the most accurately estimated parameter it is independent of parameters involving the parents (σ_0^2 and c_{01}) and in large diallel crosses is also independent of the other parameters, σ_1^2 and c_{11}.

Another method of estimating the statistical parameters is by maximum likelihood. In a bivariate normal population the expectation and the maximum likelihood methods give estimators differing only by the factor $(n-1)/n$. In our multivariate case the differences are also of this order but are more complicated in form and are worth investigating.

Let t_{ij} ($i,j = 1,2, - - - - - n$ and $t_{ij} = t_{ji}$) be the $\frac{1}{2}n(n+1)$ variables in the characteristic function corresponding to the observable variables x_{ij}. If error variances are ignored this function is

$$Q = \sigma_0^2 \Sigma t_{ii}^2 + \tfrac{1}{2}\sigma_1^2 \Sigma t_{ij}^2 + 2c_{01}\Sigma t_{ii}t_{ij} + c_{11}\Sigma t_{ij}t_{ik}$$

summation again being over-all values of the subscripts for which $i \neq j \neq k$. We can also write

$$Q = \mathbf{t}'\mathbf{V}\mathbf{t}$$

where $\mathbf{t}$ is the vector of variables t_{ij} and $\mathbf{V}$ is the variance matrix. If $\mathbf{x}$ and μ are the vectors of the variables x_{ij} and their means the joint frequency function of the variables x_{ij} is

$$(2\pi)^{-\frac{1}{4}n(n+1)}|\mathbf{V}|^{-\frac{1}{2}}\exp[-\tfrac{1}{2}(\mathbf{x}'-\mu')\mathbf{V}^{-1}(\mathbf{x}-\mu)]$$

The maximum likelihood estimators of the six parameters maximize this function or, using the negative logarithm, minimize

$$\log|\mathbf{V}| + (\mathbf{x}'-\mu')\mathbf{V}^{-1}(\mathbf{x}-\mu)$$

It can be shown that

$$\begin{aligned}|\mathbf{V}| &= (\sigma_1^2-2c_{11})^{\frac{1}{2}n(n-3)}[\sigma_0^2\sigma_1^2 - 4\sigma_0^2c_{11} + 2c_{01}^2 + 2n(\sigma_0^2c_{11} - c_{01}^2)] \\ &\quad \times [\sigma_0^2\sigma_1^2 - 4\sigma_0^2c_{11} + 2c_{01}^2 + n(\sigma_0^2c_{11} - c_{01}^2)]^{n-1} \\ &= \sigma_2^{n(n-3)}pq^{n-1}, \text{ say,}\end{aligned}$$

and that

$$\begin{aligned}&(\mathbf{x}' - \mu')\mathbf{V}^{-1}(\mathbf{x} - \mu) \\ &= [2(n-1)(\sigma_3^2+nc_{11})(x-n\mu_0)^2 - 4(n-1)c_{01}(x-n\mu_0)(X-n(n-1)\mu_1) \\ &\qquad + \sigma_0^2(X-n(n-1)\mu_1)^2]/2n(n-1)p \\ &\quad + [\sigma_3^2S_0 - 2c_{01}S_{01} + \sigma_0^2S_{11}]/q + S_1/\sigma_2^2\end{aligned}$$

where $\sigma_3^2 = \sigma_1^2 + (n-4)c_{11}$

Equating to zero the partial differentials of the likelihood with respect to each parameter we find that the estimators of the parameters are;

$$\begin{aligned}\hat{\mu}_0 &= x/n, \text{ as before} \\ \hat{\mu}_1 &= X/n(n-1), \text{ as before} \\ \hat{\sigma}_0^2 &= S_0/n \\ \hat{c}_{01} &= S_{01}/n(n-2) \\ n(n-1)(n-2)^3S_0\hat{\sigma}_1^2 &= S_0[(n-2)(3n^2-15n+22)S_{11} + (2n^2-9n+6)S_1] \\ &\quad - (n-3)(n-6)S_{01}^2 \\ &\quad - (n-6)\sqrt{[n-2)^2((n-3)^2S_{11}^2 - 2(n-1)S_1S_{11} + S_1^2)S_0^2}\end{aligned}$$

$$-2(n-2)\{(n-3)^2S_{11}-(n-1)S_1\}S_0S_{01}^2$$
$$+(n-3)^2S_{01}^4]$$
$$2n(n-1)(n-6)\hat{c}_{11}=4(n-1)S_{11}+2nS_1-n(n-1)(n+2)\hat{\sigma}_1^2$$
$$\hat{\sigma}_2^2=\hat{\sigma}_1^2-2\hat{c}_{11}$$

In the formula for $\hat{\sigma}_1^2$ the negative root is to be used as indicated.

The estimators of σ_1^2, c_{11} and σ_2^2 may be expanded as power series in $1/n$. To the third order we have

$$\hat{\sigma}_1^2=2[(n^2+2n+4)S_{11}+(n-1)(n+2)S_1]/n^4$$
$$\hat{c}_{11}=[(n^2+2n+4)S_{11}-(2n+11)S_1]/n^4$$
$$\hat{\sigma}_2^2=2(n^2+3n+9)S_1/n^4$$

The maximum likelihood estimators are the same as the unbiassed estimators to the first order in n.

The expectations of the maximum likelihood estimators (or their approximations to the second order in n) are

$$\epsilon\hat{\mu}_0=\mu_0$$
$$\epsilon\hat{\mu}_1=\mu_1$$
$$\epsilon\hat{\sigma}_0^2=(n-1)\sigma_0^2/n$$
$$\epsilon\hat{c}_{01}=(n-1)c_{01}/n$$
$$\epsilon\hat{\sigma}_1^2=(n\sigma_1^2-2c_{11})/n$$
$$\epsilon\hat{c}_{11}=(n-1)c_{11}/n$$
$$\epsilon\hat{\sigma}_2^2=\sigma_2^2$$

The biasses of the estimators of σ_0^2, c_{01} and c_{11} are of the expected nature but $\hat{\sigma}_2^2$ is approximately unbiassed and the bias of $\hat{\sigma}_1^2$ depends on c_{11}.

The sampling variances and covariances of the maximum likelihood estimators are very much more complicated than those of the unbiassed estimators but they are identical to the first order in n. To the second order in n the variance and the covariances of both estimators of σ_2^2 are the same but the other variances and covariances of the maximum likelihood estimators are less by a factor $(n-1)/n$ than their corresponding entries in Table 1. The exact sampling variances and covariances of the maximum likelihood estimators may be obtained from us. The previous discussion of the significance of unbiassed estimators, of their sampling correlations and of the minimum size of experiment applies equally to these maximum likelihood estimators. For the remainder of the paper we shall restrict ourselves to the simpler unbiassed estimators.

Genetical components of variation

HAYMAN (1954a) defined five components of genetic variation in a diallel system exhibiting additive and dominance, but no epistatic, variation. These were

$$h=\Sigma h_a(1-w_a^2)$$
$$D=\Sigma d_a^2(1-w_a^2)$$
$$F=2\Sigma d_a h_a w_a(1-w_a^2)$$
$$H_1=\Sigma h_a^2(1-w_a^2)$$
$$H_2=\Sigma h_a^2(1-w_a^2)^2,$$

where $a=1,2,---k$ denotes the k genes controlling the character measured in

the diallel table, d_a is half the difference in effect between the homozygotes of gene a, h_a is the deviation of the heterozygote of gene a from the midhomozygote and $w_a = u_a - v_a$, u_a and v_a being the frequencies of the two alleles of gene a.

Previously we described the diallel table in statistical terms as a sample from a certain multivariate normal distribution. In genetical terms, as KEMPTHORNE (1956) points out, the F_1 families of the diallel cross behave as a sample from a random mating population and the inbred lines as a sample from the same population inbred without selection. This means that w_a is the same in both parents and F_1 so that we can express the statistical components of variation in terms of the above genetic components.

The expected difference between parent and offspring means is

$$\mu = \tfrac{1}{2}h$$

The expected variance of the inbred lines is

$$\sigma_0^2 = D$$

The expected variance of the random mating population is

$$\sigma_1^2 = \tfrac{1}{2}D - \tfrac{1}{2}F + \tfrac{1}{2}H_1 - \tfrac{1}{4}H_2$$

and we also have

$$c_{01} = \tfrac{1}{2}D - \tfrac{1}{4}F$$

and $$c_{11} = \tfrac{1}{4}D - \tfrac{1}{4}F + \tfrac{1}{4}H_1 - \tfrac{1}{4}H_2$$

Hence unbiassed estimators of the genetic components of variation in the sampled population are:

$$n(n-1)\hat{h} = 2X - 2(n-1)x$$

$$(n-1)\hat{D} = S_0 - (n-1)\hat{E}_0$$

$$(n-1)(n-2)\hat{F} = 2(n-2)S_0 - 4S_{01} - 2(n-1)(n-2)\hat{E}_0$$

$$n(n-1)(n-2)\hat{H}_1 = n[(n-2)S_0 - 4S_{01} + 4S_{11}] + 8(n-1)S_1 - n(n-1)(n-2)(\hat{E}_0 + 4\hat{E}_1)$$

$$n(n-3)\hat{H}_2 = 8S_1 - 4n(n-3)\hat{E}_1$$

Since the expectation of $\hat{h}^2$ is not h^2 an unbiassed estimator of h^2 may be required. It is

$$[4(n-2)(n-3)(X-(n-1)x)^2 - 4n(n-1)(n-3)((n-2)S_0 - 4S_{01} + 4S_{11}) + 16n(n-1)S_1]/n^2(n-1)^2(n-2)(n-3)$$

Other models of genetic behavior might be considered and as long as these determine not more than five components of genetic variation estimates of these components can be found in terms of the five statistical components of variation in the diallel table.

The assumptions (HAYMAN 1954a) behind HAYMAN and JINKS' model of genetic variation in the diallel cross imply that when the diallel cross is considered, as in the present case, to be derived from a population then that population is random mating and free from epistasis. Now MATHER (1949) showed that the genetic portion of all variances and covariances, however related, from such a random mating population could be expressed in terms of just two components (our notation),

$$D' = \Sigma(d_a - h_a w_a)^2(1 - w_a^2)$$

and $$H' = \Sigma h_a^2(1 - w_a^2)^2$$

Thus $D' = 4c_{11} = 4\sigma_g^2$
and $H' = 4\sigma_1^2 - 8c_{11} = 4\sigma_2^2 = 4\sigma_s^2$
where σ_g^2 and σ_s^2 are the components of variation in general and specific combining ability estimated by

$$\hat{\sigma}_g^2 = [n(n-3)S_{11} - 2(n-1)S_1]/n(n-1)(n-2)(n-3)$$

and $\hat{\sigma}_s^2 = 2S_1/n(n-3) - \hat{E}_1$

As GRIFFING (1956a) points out only the F_1 families are needed to estimate σ_g^2 and σ_s^2 (or MATHER's D' and H').

Just what then is the extra information supplied by the parents, and how important is it? H' is a measure of dominance influenced to some extent by asymmetry of the gene distributions ($w_a \neq 0$) in the population. D' depends on the terms $d_a - h_a w_a$ which measure the average increment in the character when a gene substitution is made in the whole population at locus a. This increment is the only measure of additive gene action available in a random-mating population but it depends on dominance and gene frequency as well. On the other hand, d_a and h_a are independent of each other and of any population as long as epistasis is absent (HAYMAN 1958b).

The importance of the parents to the diallel cross is that they permit the separation of d_a and h_a in D' or, in other words, enable the true additive and dominance components of general combining ability to be separated. By including the parents, D' and H' can be replaced by the five components h^2, D, F, H_1 and H_2.

As one example of information not supplied by D' and H' or by the combining abilities we take the measure of mean degree of dominance,

$$\frac{H_1}{D} = \frac{\Sigma h_a^2(1-w_a^2)}{\Sigma d_a^2(1-w_a^2)}$$

Although gene frequency does enter this expression it does not thereby confound additive and dominance effects as in D'. Moreover, provided that w_a^2 is not correlated with degree of dominance,

$$\frac{\epsilon H_1}{\epsilon D} = \frac{\Sigma h_a^2}{\Sigma d_a^2}$$

so that on the average H_1/D measures mean degree of dominance independently of gene frequency.

Other properties of the random-mating population can be inferred from h^2, F and H_2, a full account being given by HAYMAN (1954a). This account actually concerned a fixed set of inbred lines but when the inbred lines are derived from a random-mating population it applies to the population itself as long as the correct estimators of the components are used.

Table 3 contains the sampling variances and covariances of the genetic components of variation. Just as Table 2 was derived from Table 1 to exhibit the significance of the estimators of the statistical components of variation and the correlations between them, so a similar table may be constructed from Table 3 for the genetic components. Such a table shows, as before, that at least ten parents

TABLE 3

Variance matrix of unbiassed estimators of genetical components

	$\hat{D}$	$\hat{F}$	$\hat{H}_1$	$\hat{H}_2$	$\hat{h}^2$
$\hat{D}$	$\frac{2v_0^2}{n\text{-}1}+\frac{2E_0^2}{n_0}$	$\frac{4v_0v_{01}}{n\text{-}1}+\frac{4E_0^2}{n_0}$	$\frac{2v_{01}^2}{n\text{-}1}+\frac{2E_0^2}{n_0}$	0	$\frac{-8v_{01}^2}{n(n\text{-}1)}$
$\hat{F}$		$\frac{8v_0v_4+16(n\text{-}2)c_{01}^2}{(n\text{-}1)(n\text{-}2)}+\frac{8E_0^2}{n_0}$	$\frac{4v_{01}v_5}{(n\text{-}1)(n\text{-}2)}+\frac{4E_0^2}{n_0}$	0	$\frac{-16v_{01}v_5}{n(n\text{-}1)(n\text{-}2)}$
$\hat{H}_1$			$\frac{64(n\text{-}3)v_2^2}{n(n\text{-}2)^2}+\frac{2v_5^2}{(n\text{-}1)(n\text{-}2)^2}+\frac{2E_0^2}{n_0}+\frac{32E_1^2}{n_1}$	$\frac{64v_2^2}{n(n\text{-}2)}+\frac{32E_1^2}{n_1}$	$\frac{128v_2^2-8v_5^2}{n(n\text{-}1)(n\text{-}2)^2}$
$\hat{H}_2$				$\frac{64v_2^2}{n(n\text{-}3)}+\frac{32E_1^2}{n_1}$	$\frac{128v_2^2}{n(n\text{-}1)(n\text{-}2)(n\text{-}3)}$
$\hat{h}^2$					$\frac{256nv_2^2+32(n\text{-}1)(n\text{-}3)v_5^2}{n^2(n\text{-}1)^2(n\text{-}2)^2(n\text{-}3)}+\frac{48v_6^2}{n^2(n\text{-}1)^2}$

where $v_0=E_0+\sigma_0^2$; $v_{01}=E_0+\sigma_0^2-2c_{01}$; $v_2=E_1+\sigma_1^2-2c_{11}$; $v_4=(n\text{-}2)(E_0+\sigma_0^2-4c_{01})+2(E_1+\sigma_1^2)+2(n\text{-}4)c_{11}$

$v_5=(n\text{-}2)(E_0+\sigma_0^2-4c_{01})+4(E_1+\sigma_1^2)+4(n\text{-}4)c_{11}$; $v_6=(n\text{-}1)(E_0+\sigma_0^2-4c_{01})+2(E_1+\sigma_1^2)+2(n\text{-}2)c_{11}$

Also $var\,\hat{h}^2=4v_6/n(n\text{-}1)$ and $\hat{h}^2$ is uncorrelated with the estimators of D, F, H_1 and H_2.

are required before most of the components can be estimated. The most accurately estimated component is H_2. If the environmental components are small or are very accurately estimated and if n is not too small, the estimators of H_2 and h^2 are nearly independent of the other estimators. The estimators of D, F and H_1 are intercorrelated to much the same extent as the estimators of σ_0^2, c_{01} and σ_1^2. If environmental components become important many of the correlations amongst the estimators of D, F, H_1 and H_2 are increased.

At the risk of becoming tedious we must repeat that the restriction to at least ten parents before useful information can be inferred about the sampled population does not apply to a diallel cross designed to measure the properties of a specific set of inbred lines. Indeed there is no reason why a cross between only two lines should not be considered: this is, of course, a very common type of experiment.

As a final note to this section we express the unbiassed estimators of the genetic components of variation in terms of the diallel statistics defined by HAYMAN (1954a) and JINKS (1954). These differ from the statistics used so far in this paper in including the diagonal term in each array, which practice has some computational advantages. These alternative forms are

$$\begin{aligned}
(n-1)\hat{h} &= 2n(m_{L1}-m_{L0}) \\
\hat{D} &= V_{0L0}-\hat{E}_0 \\
(n-2)\hat{F} &= 2nV_{0L0}-4nW_{0L01}-2(n-2)\hat{E}_0 \\
(n-1)(n-2)\hat{H}_1 &= (n-1)(n-2)(V_{0L0}-4W_{0L01}+4V_{0L1}) \\
&\quad +4(n-1)^2(V_{1L1}-V_{0L1})-4n(m_{L0}-m_{L1})^2 \\
&\quad -(n-1)(n-2)(\hat{E}_0+4\hat{E}_1) \\
(n-1)(n-2)(n-3)\hat{H}_2 &= -4(n-1)^2(V_{0L0}-4W_{0L01}+4V_{0L1}) \\
&\quad +4(n-1)^2(n-2)(V_{1L1}-V_{0L1})-4n(n-2)(m_{L0}-m_{L1})^2 \\
&\quad -4(n-1)(n-2)(n-3)\hat{E}_1
\end{aligned}$$

and the unbiassed estimator of $(n-1)(n-2)(n-3)h^2$ is

$$-4(n-1)^2(V_{0L0}-4W_{0L01}+4V_{0L1})+8(n-1)(V_{1L1}-V_{0L1})$$
$$+4n(n^2-4n+2)(m_{L0}-m_{L1})^2$$

Analysis of variance

The purpose of the analysis of variance of a replicated experiment is to separate components of random variation and to estimate non random effects (EISENHART 1947). In our case, only simple analyses of the variance of parent families and of F_1 families are needed to supply estimates of E_0 and E_1.

More complex analyses of variance can estimate and test the significance of specific components of variation, but since they cannot supply a complete set either of estimators or of tests of significance, they have limited value. In any case our unbiassed estimators are identical to these estimators, where comparable, and the tests of significance agree to the first order in n.

Significance levels in the analysis of variance should be interpreted with caution. High significance implies that accurate estimates of means can be obtained. It does not necessarily imply accurate estimation of the variance of these means, that is to say, the variance components are not necessarily accurate measures of

their corresponding parameters in the sampled population. To take a simple example, a replicated experiment containing just two lines may demonstrate a definite genetic difference between the lines but, no matter how accurately this genetic difference may be determined, it cannot be an accurate measure of the genetic variation in the population from which the two lines have been sampled. As we have seen, a sample of at least ten is needed for reasonably accurate estimation of population variability. On the other hand, lack of significance of a particular component in the analysis of variance does imply that it has no significant estimate but the standard errors of Tables 1 and 3 will lead to the same conclusion.

For completeness, we present the restricted results supplied by the analysis of variance. When the inbred lines are derived from a population, the expectations of HAYMAN's (1954b) mean squares for the diallel table of family means are, ignoring reciprocal differences,

$$n\epsilon a = 2(E_0+\sigma_0^2) + 4(n-2)c_{01} + 2(n-2)(E_1+\sigma_1^2) + 2(n-2)(n-4)c_{11}$$
$$n\epsilon b_1 = (n-1)(E_0+\sigma_0^2) - 4(n-1)c_{01} + 2(E_1+\sigma_1^2) + 4(n-2)c_{11} + n(n-1)\mu^2$$
$$n\epsilon b_2 = (n-2)(E_0+\sigma_0^2) - 4(n-2)c_{01} + 4(E_1+\sigma_1^2) + 4(n-4)c_{11}$$
$$\epsilon b_3 = 2(E_1+\sigma_1^2) - 4c_{11}$$

These four equations cannot provide estimates of five parameters, as HAYMAN (1954b) has stated, but some combinations of the parameters can be estimated. Two such are

$$(n-1)(n-2)\hat{\mu}^2 = (n-2)b_1 - (n-1)b_2 + b_3$$
$$2\hat{\sigma}_1^2 - 4\hat{c}_{11} = 2\hat{\sigma}_2^2 = b_3 - 2\hat{E}_1$$

Three genetical components of variation can be estimated,

$$(n-2)\hat{H}_1 = nb_2 + 2(n-3)b_3 - (n-2)(\hat{E}_0+4\hat{E}_1)$$
$$\hat{H}_2 = 2b_3 - 4\hat{E}_1$$
$$(n-1)(n-2)\hat{h}^2 = 4(n-2)b_1 - 4(n-1)b_2 + 4b_3.$$

Further, b_3 provides an exact test of significance of H_2 and combinations of b_1, b_2, b_3, $\hat{E}_0$ and $\hat{E}_1$ provide approximate tests of significance (COCHRAN 1951) of H_1 and h^2. The difference $(n-2)(\hat{H}_1-\hat{H}_2) = nb_2 - 2b_3 - (n-2)\hat{E}_0$ also provides a useful test.

GRIFFING (1956a,b, 1958) obtained from the F_1 families alone the two mean squares, S_{11} and S_1 in our notation, which supply estimates of σ_1^2 and c_{11}.

Example

DR. G. F. SPRAGUE has kindly supplied the yields from four blocks of a maize diallel cross between ten lines. HAYMAN (1958a) analysed these data assuming that the ten parents comprised the total population under consideration, but here we assume that the parents are a random sample of ten inbred lines from a population.

The preliminary analyses of parental and F_1 yields reveal differences between parents and between crosses significant at a probability level of about 10^{-10} in both cases. Evidently genetic variation is present so that we can proceed to esti-

mate the population parameters. The basic statistics, in terms of totals over blocks, are

$x = 4591$ $S_0 = 443{,}705$ $E_0 = 1177$
$X = 118{,}370$ $S_{01} = 1161{,}160$ $n_0 = 30$
$n = 10$ $S_{11} = 813{,}892$ $E_1 = 8445$
$SS_1 = 839{,}780$ $n_1 = 135$

The estimates and standard errors of the means are

$$\hat{\mu}_0 = 459 \pm 70$$
$$\hat{\mu}_1 = 1315 \pm 62$$
$$r(\hat{\mu}_0, \hat{\mu}_1) = 0.740$$

The unbiassed estimators of the statistical components of variation, together with their sampling errors and correlations, are in Table 4. Comparison of the

TABLE 4

Unbiassed estimators of statistical components

	σ_0^2	c_{01}	σ_1^2	c_{11}	σ_2^2
Estimate	48,124	16,127	32,158	8,305	15,549
Standard error	23,243	9,530	11,539	5,377	5,827
Correlations σ_0^2		0.797	0.431	0.462	0.000
c_{01}			0.737	0.791	0.000
σ_1^2				0.866	0.383
c_{11}					—0.131

estimates with their standard errors shows that σ_1^2 and the derived parameter σ_2^2 are highly significant, that σ_0^2 is just significant and that the covariances c_{01} and c_{11} are not significant. The estimator of σ_2^2 is almost independent of the other estimators while amongst these c_{01}, if significant, would be highly correlated with the other three while σ_1^2 and c_{11} would be even more highly correlated.

The maximum likelihood estimators, corrected for error variance, their correlations and the third order approximations to the last three estimators are in Table 5. These results, including the approximations, are consistent with Table 4.

Table 6 contains similar information about the genetic model of HAYMAN and JINKS, assuming it to apply. HAYMAN (1958a), by considering F_2 families as well, showed that epistasis was present but we ignore it for the purpose of illustrating our methods of estimation. The dominance components, H_1, H_2 and h^2 are highly significant, the additive component D is significant and F is not significant.

The analyses of variance confirm these results as far as they can. HAYMAN's shows high significance of H_1, H_2 and h^2 and GRIFFING's of c_{11} and σ_2^2. In every case significant unbiassed estimators are also significant in the analysis of variance. c_{11} is an example of the failure of the converse. Apparently there are differences between the ten parents in general combining ability but these do not

TABLE 5

Maximum likelihood estimators of statistical components

		σ^2_0	c_{01}	σ^2_1	c_{11}	σ^2_2
Estimate		43,194	14,514	29,649	6,881	15,886
Approximation		....	....	29,879	7,489	14,901
Standard error		19,845	8,002	9,800	4,368	5,891
Correlations	σ^2_0		0.811	0.433	0.486	0.000
	c_{01}			0.717	0.804	0.000
	σ^2_1				0.804	0.471
	c_{11}					—0.146

TABLE 6

Unbiassed estimators of genetic components

		D	F	H_1	H_2	h^2
Estimate		48,124	31,738	79,029	62,195	2,922,444
Standard error		23,243	28,054	26,167	23,308	22,234
Correlations	D		0.573	0.106	0.000	—0.050
	F			0.356	0.000	—0.163
	H_1				0.783	—0.160
	H_2					0.028

provide a good measure of the variability in general combining ability in the sampled population. GRIFFING's (1956a) Tables 12 and 13 show the same contrast. In his analysis of variance general combining ability differences are highly significant, but his estimates of σ^2_g are well under twice their standard errors. Again, HAYMAN's analysis of variance shows that the difference H_1—H_2 is just significant, but no useful measure of this difference (and therefore of gene asymmetry in the population) can be obtained from our Table 6. Some components, of course, are not tested at all in these analyses of variance.

One difference between our present analysis in terms of sampled parents and that of HAYMAN and JINKS in terms of fixed parents should be noted. Our estimate of the measure of average degree of dominance $(H_1/D)^{\frac{1}{2}}$ is 1.28 which is not significantly greater than unity while, on the assumption that the ten lines comprise the total inbred population under consideration, the estimate is 2.75 and significantly greater than unity. HAYMAN (1958a) considered a nonepistatic subdiallel cross of six lines and for this the two estimates are respectively 3.46 and 8.75. The differences, by over a factor or two, between these pairs of values are of the same nature as the difference between the unbiassed estimate of a population variance from a sample, $\Sigma(x_i-\bar{x})^2/(n-1)$, and the actual variance of the sample, $\Sigma(x_i-\bar{x})^2/n$. However, in the case of our H_1, the difference is so

great that it suggests care should be exercised in inferring overdominance from the analysis of a fixed set of inbred lines.

SUMMARY

Diallel crosses between inbred lines have several experimental uses. A distinction must be made between measuring the properties of the lines themselves and the properties of the population from which the lines are considered to be a sample, the former case having been discussed in papers by HAYMAN and JINKS, the latter case of KEMPTHORNE and both cases by GRIFFING. Another distinction is between the genetic components to be estimated, HAYMAN and JINKS having developed estimators of additive and various kinds of dominance components and the other authors estimators of general and specific combining abilities.

In this paper HAYMAN and JINKS' analysis of a fixed set of inbred lines has been extended to sampled inbred lines. Their five components of additive and dominance variation are related to the two combining ability components and the extra information in the former is demonstrated.

Two general methods of estimation, by unbiassed estimators and by maximum likelihood, are described, the former being the simpler and differing little from the latter numerically. At least ten parents should be used for reasonably accurate estimation of components of variation.

The analysis of variance provides estimates of only some of the components. Furthermore, while it is useful in demonstrating genetic variation, it does not provide suitable significance levels for the components. These are determined by the other methods of estimation.

An analysis of maize yields revealed additive and dominance variation. There was also some suggestion of gene asymmetry in the sampled population. Although a previous analysis suggested that these ten lines display overdominance amongst themselves the present analysis provides no evidence for this in the population from which the lines were sampled.

LITERATURE CITED

COCHRAN, W. G., 1951 Testing a linear relation among variances. Biometrics **7**: 17–32.

EISENHART, C., 1947 The assumptions underlying the analysis of variance. Biometrics **3**: 1–21.

GRIFFING, B., 1956a Concept of general and specific combining ability in relation to diallel crossing systems. Australian J. Biol. Sci. **9**: 463–493.

1956b A generalised treatment of the use of diallel crosses in quantitative inheritance. Heredity **10**: 31–50.

1958 Application of sampling variables in the identification of methods which yield unbiassed estimates of genotypic variance components. Australian J. Biol. Sci. **11**: 219–245.

HAYMAN, B. I., 1954a The theory and analysis of diallel crosses. Genetics **39**: 789–809.

1954b The analysis of variance of diallel tables. Biometrics **10**: 235–244.

1957 Interaction, heterosis and diallel crosses. Genetics **42**: 336–355.

1958a The theory and analysis of diallel crosses. II. Genetics **43**: 63–85.

1958b The separation of epistatic from additive and dominance variation in generation means. Heredity **12**: 371–390.

JINKS, J. L., 1954 The analysis of continuous variation in a diallel cross of *Nicotiana rustica* varieties. Genetics **39**: 767–788.

1956 The F_2 and backcross generations from a diallel cross of *Nicotiana rustica* varieties. Heredity **10**: 1–30.

KEMPTHORNE, O., 1956 The theory of the diallel cross. Genetics **41**: 451–459.

MATHER, K., 1949 *Biometrical Genetics.* Methuen and Co. London.

MATZINGER, D. F., and O. KEMPTHORNE, 1956 The modified diallel table with partial inbreeding and interactions with environment. Genetics **41**: 822–833.

NELDER, J. A., 1953 Statistical models in biometrical genetics. Heredity **7**: 111–119.

YATES, F., 1947 Analysis of data from all possible reciprocal crosses between a set of parental lines. Heredity **1**: 287–301.

GENETIC STUDIES OF SERUM ANTIGENS IN SPECIES HYBRIDS[1]

S. L. SCHEINBERG[2]

Department of Genetics, University of Wisconsin
Madison, Wisconsin

Received July 13, 1959

STUDIES of serum proteins in taxonomy had their beginnings with the classical work of NUTTALL (1904) who used the antigenic properties of sera as criteria for classification. Subsequently, numerous studies were made of serum differences between species. In these studies *in vitro* precipitation of serum-antiserum mixtures was employed to distinguish sera either by the degree of flocculation or the presence or absence of an interfacial ring. The results of these investigations were summarized by LANDSTEINER and VAN DER SCHEER (1924, 1940). The differences found in the sera of species were quantitative in that antisera reacted more strongly with the serum of the homologous than of heterologous species.

Relatively few studies were made in which the serum antigens of one species could be sharply differentiated from those of another. SASAKI (1937) reported that he was able to differentiate the serum of the White Leghorn chicken (*Gallus gallus*) from that of the guinea fowl (*Numida meleagris*) by absorbing antisera to one species with the serum of the heterologous species. According to SASAKI (1935) similar results had been obtained previously by ISHIHARA and MISAO in their studies of carp and by SASAKI in an investigation of a species cross in ducks.

In a series of papers CUMLEY and IRWIN (1940, 1942, 1943, 1952) reported that segregation of serum antigens occurs among backcross progeny of species and generic hybrids in accordance with an hypothesis of unifactorial inheritance. The genes responsible for the serum specificities are not the same as the causative genes for the differentiating cellular substances and may be independent of or loosely linked to these genes. More recently there have been a number of intraspecific studies implicating genes in the formation or properties of serum proteins. THOMPSON, FOSTER, GOWEN, and TAUBER (1954) reported differences among strains of mice with respect to the globulin component as determined electrophoretically. There were also differences among the mouse strains with respect to the total serum protein content. A protein with hemoglobin binding properties, i.e. haptoglobin, has been studied in man by SMITHIES and WALKER (1956). They presented family data to support their hypothesis that the synthesis of the haptoglobins is under the control of a two allele system. Evidence in support of this hypothesis has also been obtained by F. GALATIUS-JENSEN (1957). Genetic control of protein synthesis is also indicated in the suggestive evidence that the

[1] From the Department of Genetics (No. 722), University of Wisconsin, research conducted at the University of Wisconsin. Formerly Postdoctoral Fellow of the National Institutes of Health.

[2] Present address—Agricultural Research Service, Animal Husbandry Division, U.S. Dept. Agriculture, Beltsville, Maryland.

absence of globulin in man (agammaglobulinemia) is an inherent metabolic error, GITLIN, HITZIG, and JANEWAY (1956). Genetic variation may also account for the differences among individual rabbits in the antigenic components of serum observed by DRAY and YOUNG (1958).

The present paper deals with analyses of the sera of species among the Bovidae and Columbidae. Differences between species, species hybrids and backcross hybrids were detected with specific antisera in flocculation tests. Electrophoretic analyses were also made of the sera of species and their hybrids. These studies provide additional evidence for the genetic control of antigenic specificity among the serum antigens.

MATERIALS AND METHODS

Samples of sera were obtained from cattle (*Bos taurus*), bison (*Bos bison*), pigeons (*Columba livia*), ringdoves (*Streptopelia risoria*), dwarf turtledoves (*Streptopelia humilis*, or *tranquebarica* hereinafter referred to as *humilis*) and hybrids and backcross hybrids of the following species crosses: bison × cattle; ringdove × *humilis* and ringdove × pigeon. In the Bovidae, cattle were the recurrent species used to obtain the backcross progeny, in the other matings the ringdove was the recurrent parental species.

Blood samples from the Bovidae were obtained by jugular puncture and from the Columbidae by brachial venipuncture. Serum was obtained from the clotted blood and stored at −20°C until used. In order to reduce the lipid content of the sera of birds and rabbits the animals were starved for 24 hours before bleeding. Prior to immunization the sera of the rabbits were tested for the presence of naturally occurring antibodies. Such antibodies were found to a titer of two to three ($\log_2$) for both cattle and bison and also for *humilis*, pigeon and ringdove. Eighteen rabbit normal sera were tested with the sera of Columbidae and six rabbit normal sera with the sera of the Bovidae.

All immunizations were made with the pooled sera or plasma with the number included in each pool as follows: bison-93, ringdove-100, *humilis*-30, pigeon-50. Injections were made with a total of 6 cc of fluid, 0.2 cc for the first injection and 0.4 cc every other day until the series was completed. Ten days following the final injection the rabbits were bled providing the titer was five ($\log_2$) or greater. Blood was obtained from the marginal ear vein early in the day and a second bleeding was made intracardially later in the same day and the samples of sera were pooled. This procedure resulted in greater yields of blood than the use of either method alone.

All fluids used in the flocculation tests were preserved with the addition of merthiolate 1:3000. Tubes were made up with equal quantities of antigen and antibody solution, incubated at 37°C and scored macroscopically. Preliminary flocculation tests were carried out to determine the optimal proportion of antigen to antibody at which absorptions were performed. Antisera were exhaustively absorbed until the heterologous antigen solution failed to react with the antisera. Mixtures of antigen and antibody were incubated at 37°C for periods of one to

two hours and then centrifuged for 15 minutes at 2500 rpm in a refrigerated centrifuge. In successive absorptions the amount of antigen solution used for absorption was halved until the antisera failed to react with the absorbing antigen.

Free electrophoresis was carried out in the Spinco electrophoresis apparatus using the Alberty cell (ALBERTY 1948). The duration of the run was 10,800 seconds and the imposed current was 16 milliamperes across a potential difference of 250 volts. Prior to electrophoresis the sera were dialyzed at 4°C for 24 hours against sodium veronal, pH 8.6 and 0.1 ionic strength. The concentration of protein was initially determined with the use of a Bausch and Lomb dipping refractometer. Buffer conductivity was measured with a Wheatstone bridge assembly for each run. Measurements for mobility and relative concentration were made on the descending portion of the electrophoretic pattern in the case of the bison and bovine sera. Since all bird sera examined possessed the β anomaly in the descending pattern which precluded the use of this pattern in the analysis, the ascending pattern was used. The relative proportions of each serum component were determined from the areas as measured with a planimeter. Each component was delimited so as to form a gaussian distribution. The procedure of SMITHIES and WALKER (1956) was used in making the starch gel electrophoresis runs.

RESULTS

Serum antigens differentiating humilis *and ringdove*

In flocculation tests anti-*humilis* sera reacted strongly with the serum antigens of ringdove as well as with the homologous sera of *humilis*. The sera of the two species share specificities but can be differentiated following absorption. The $\log_2$ of antigen titer, that is, the titration of antigen with constant antibody concentration averaged nine prior to absorption for the 11 sera or plasma and averaged five following absorption. The corresponding values for antibody titer were 5.4 and 1.3.[3] These data are summarized in Table 1.

Paired comparisons of absorbed antisera for their reactivity with the sera of a given group of hybrids and backcross hybrids were made to determine whether any two or more of the antisera were identical. Identical antisera would be expected to show complete correspondence in reactivities when comparisons would be made of a given hybrid serum with the two antisera in question whereas noncorrespondence would indicate nonidentity of the antisera. Two antisera which showed a high percentage of correspondence would be presumed to share antibody specificities or to possess some measure of cross-reactivity, or both. In all the comparisons made there were none in which there was complete correspondence. There were 28 comparisons, 25 of which showed a noncorrespondence of 20 percent or greater, the remaining five did not correspond in 7, 13, 13, 14 and 18 percent of comparisons, respectively (Table 2). The correspondence of the latter comparisons was not restricted to one or two antisera but included five of the eight antisera tested. It is concluded that no two of the antisera were identical, and all will be treated as if they detect different antigenic substances in the serum.

TABLE 1

Titres (to log of base) of sera and plasma prior to and following absorption with pooled sera of ringdove

No. of serum and plasma	Antigen titer		Antibody titer	
	Before	After absorption	Before	After absorption
anti-*humilis*				
$37F_3$	9	5	6	1
$42F_1$	9	4	6	1
$43F_1$	9	5	5	2
$48F_2$	9	5	5	1
$48F_3$	9	5	5	2
$51F_3$	6	4	6	1
$947F_1$	10	6	7	1
$949F_2$	10	6	5	1
$961F_1$	10	–	5	1
$961F_5$	10	7	5	2
$965F_1$	10	5	5	2
antiringdove (not absorbed)				
$956F_1$	7	.	6	.
$962F_4$	7	.	6	.
$965F_2$	7	.	6	.
antipigeon				
$36F_5$	10	5	–	–
$42F_4$	8	5	–	–
$43F_4$	8	5	–	–
$48F_1$	8	7	–	–
$51F_1$	8	5	–	–
$51F_3$	–	–	8	5
$959F_1$	10	7	5	1
$961F_6$	–	–	–	–

– indicates test was not performed.

Future studies should indicate whether any of the antisera share antibody specificities.

The absorbed fluids were tested with the sera of the *humilis* × ringdove hybrids and backcross hybrids to determine whether the antigens were distributed according to Mendelian expectation. These results are summarized in Table 3. The reactions of individual hybrids are included in detailed tables which will be furnished upon request[3]. The sera of the F_1 hybrids, except two for three antisera and one for three other antisera, reacted with the *humilis* specific antisera as expected if the *humilis* parental species has contributed genes to the hybrid which are responsible for the serum specificities which distinguish the *humilis* serum from the ringdove serum. If more than one independent gene contributed by the *humilis* parental species is responsible for the antigenic differences between the species, then the first backcross generation is expected to contain more birds with

[3] Detailed data for which reference is made in the text will be furnished on request.

reactive sera than birds lacking the serum antigens. These would be in the general proportion of 2^n-1 reactive to one nonreactive bird where "n" is the number of independent genes and the antisera cannot be used to discriminate between the antigenic products of the genes. In subsequent backcross generations the number of progeny whose sera are not reactive will increase depending on the number of *humilis* genes present and the genetic constitution of the parents available to form the next backcross generation. The number of progeny whose sera are nonreactive with *humilis* specific antisera No. $947F_1$, $949F_2$, $961F_1$ and $961F_5$ increases steadily with each successive backcross generation as would be expected with the continuous replacement of *humilis* genes with ringdove genes.

The data obtained from the reactions of the sera of backcross families possessing both reactive and nonreactive progeny were tested under the hypothesis that segregation had occurred with a ratio of one reactive to one nonreactive progeny,

TABLE 2

*Degree of identity of anti-*humilis *sera**

Percent noncorrespondence (number of sera compared, in parenthesis)	Antiserum designation							
	$37F_3$	$43F_1$	$48F_2$	$48F_3$	$947F_1$	$949F_2$	$961F_1$	$961F_5$
37 (43)	X	X	....	....	....	....	....	....
40 (25)	X	....	X	....	....	....	....	....
26 (27)	....	X	X	....	....	....	....	....
32 (19)	X	....	....	X	....	....	....	....
24 (21)	....	X	....	X	....	....	....	....
13 (23)	....	....	X	X	....	....	....	....
45 (20)	X	....	....	....	X	....	....	....
35 (20)	....	X	....	....	X	....	....	....
27 (11)	....	....	X	....	X	....	....	....
27 (11)	....	....	....	X	X	....	....	....
52 (29)	X	....	....	....	....	X	....	....
57 (30)	....	X	....	....	....	X	....	....
24 (17)	....	....	X	....	....	X	....	....
18 (11)	....	....	....	X	....	X	....	....
22 (47)	....	....	....	....	X	X	....	....
64 (11)	X	....	....	....	....	....	X	....
30 (10)	....	X	....	....	....	....	X	....
20 (10)	....	....	X	....	....	....	X	....
20 (10)	....	....	....	X	....	....	X	....
24 (17)	....	....	....	....	X	....	X	....
7 (14)	....	....	....	....	....	X	X	....
60 (20)	X	....	....	....	....	....	....	X
42 (21)	....	X	....	....	....	....	....	X
35 (20)	....	....	X	....	....	....	....	X
31 (16)	....	....	....	X	....	....	....	X
14 (21)	....	....	....	....	X	....	....	X
27 (30)	....	....	....	....	....	X	....	X
13 (16)	....	....	....	....	....	....	X	X

* The comparison of two antisera is indicated by the presence of two "X's" on the same horizontal line.

TABLE 3

*Summary of flocculation tests of specific anti-*humilis *sera or plasma with the sera of* humilis-*ringdove hybrids and backcross hybrids*

	(Absorbed with sera of ringdove)*															
	Anti-*humilis* sera								Anti-*humilis* plasma*							
Group	$37F_3$		$43F_1$		$48F_2$		$48F_3$		$947F_1$		$949F_2$		$961F_3$		$961F_5$	
	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0
F_1	7	2	8	2	9	1	9	0	7	1	4	1	6	2	6	0
1st Backcross	3	3	3	4	4	3	3	4	10	0	10	0	8	1	10	0
2nd Backcross	3	8	5	7	8	4	3	4	–	–	7	3	2	0	10	4
3rd Backcross	0	10	3	7	1	0	1	0	6	9	13	4	2	0	3	3
4th Backcross	0	8	3	5	–	–	–	–	7	4	7	4	–	–	–	–

\+ indicates progeny whose sera are reactive.
0 indicates progeny whose sera are not reactive.
– indicates tests were not performed.
* For all these antisera, the pooled sera of *humilis* were reactive and the sera used for absorption, that of ringdoves, were not reactive.

TABLE 4

A priori *test of hypothesis of 1:1 segregation among backcross hybrids of* humilis *with ringdove and bison and cattle*

Designation of antiserum	Number of families	Expected		Observed		Probability of greater value of χ^2
		With reactive sera	With nonreactive sera	With reactive sera	With nonreactive sera	
			Anti-*humilis*			
$37F_3$	3	7.0	8.0	6.0	9.0	<.7>.5
$43F_1$	7	10.4	13.6	12.0	12.0	<.7>.5
$48F_2$	5	7.3	9.7	11.0	6.0	<.1>.05
$48F_3$	4	5.1	6.9	6.0	6.0	<.7>.5
$947F_1$	4	4.5	6.5	4.0	7.0	<.7>.5
$949F_2$	5	8.5	10.5	11.0	8.0	<.3>.2
$961F_5$	3	6.5	7.5	8.0	6.0	<.5>.3
			Antibison			
$43F_3$	11	7.9	15.0	10.0	13.0	<.5>.3

the conventional backcross test. The *a priori* method of BERNSTEIN (STERN 1950) was used to determine the validity of the hypothesis. Table 4 lists the observed and expected numbers on an hypothesis of a one to one segregation. In all cases there was no statistically significant departure from the expected values which supports the hypothesis of single gene segregation among the backcross progeny for the serum antigens tested.

The parents of different families were not always alive at the time the progeny were tested. It has therefore been necessary to assume that the serum of the backcross hybrid parent likewise would have been reactive. In all cases but one where the backcross parents were available their sera were found to be reactive.

The one exception involved a progeny whose sera reacted faintly (score of one with a maximum possible score of seven) at one serum dilution with antiserum $949F_2$, whereas the parent serum did not react. It was not possible to test the serum of the parental hybrid at a lower serum dilution to determine whether the serum was completely without activity.

The small volume of serum which was obtained from each dove precluded the absorption of the *humilis* specific antisera with the reactive serum of each of the backcross hybrids. Consequently it is not known whether the same antigen, and hence the same gene, is involved in all the families tested with a given absorbed antiserum. With antisera $37F_3$, $43F_1$, $48F_2$ and $48F_3$ there was apparent segregation in each backcross generation so that it appears likely that only a single gene is involved. With antisera $947F_1$, $949F_2$, $961F_1$ and $961F_5$, it seems likely that more than one antigenic substance was identified by the antisera since the first and second backcross generations possess a preponderance of hybrids whose sera are reactive. It is, therefore, less likely that in these cases the same gene is segregating in each backcross family but absorption experiments should provide evidence concerning this question.

All of the antisera tested differ one from the other when comparisons of the flocculation reactions are made and imply as many different serum antigens as there are antisera. Through the kindness of PROFESSOR M. R. IRWIN who furnished data on the erythrocyte antigens it has been possible to make comparisons of serum antigens with erythrocyte antigens. The evidence available does not indicate a common gene origin for a serum antigen and a cellular antigen; the segregations in fact suggest loose linkage or independence (Table 5).

Serum antigens of pigeons and ringdoves

Additional evidence of segregation of serum antigens was obtained from flocculation tests of the sera of hybrids and backcross hybrids of pigeons and ringdoves. Seven antisera to sera or plasma of pigeons were obtained which possessed an average antigen titer ($\log_2$) of 8.6 prior to and 5.8 following absorption with serum of ringdove (Table 1). The respective antibody titers for two of the antisera which differentiated the sera of pigeon and ringdove, respectively, were eight and five prior to, and five and one following absorption.

Four of the absorbed fluids were present in sufficient quantities to test the hybrids and backcross hybrids for the presence of segregation. These results are presented in Table 6. $JTPDD_5$ serum reacted with antiserum $965F_3$ unexpectedly since that of his sire JTPD did not. Unfortunately further tests could not be made because of death of the bird. The segregations observed in the backcross hybrids confirm and extend the findings of IRWIN and CUMLEY (1942) of differences in the serum antigens between pigeons and ringdoves. As with the anti-*humilis* sera all of the antipigeon sera appeared to identify different antigens. When antiserum $42F_4$ was absorbed with the serum of each of the four reactive sera of the backcross hybrids, or in the case of $43F_4$ with the reactive sera of two of the backcross hybrids, all activity was removed for the other reactive backcross progeny

TABLE 5

Relation of serum antigens to cellular antigens in humilis-*ringdove hybrids and backcross hybrids*

Antiserum designation	Classes*				Erythrocyte antigenic factor
	+ +	+ 0	0 +	0 0	
$37F_3$	1	(1)	1	3(1)	1
$43F_1$	(1)	1(1)	3(1)	1(1)	.
$48F_2$	1(1)	2(1)	1	2(2)	.
$48F_3$	1(1)	(1)	2	3(2)	.
$51F_3$	1	0	0	1	.
$949F_2$	1	1	0	0	.
$961F_1$	0	2	1	0	.
$961F_5$	0	1	1	0	.
$37F_3$	5	2	1	6	4
$43F_1$	5	3	3	8	.
$48F_2$	8	2	4	4	.
$48F_3$	5	3	1	3	.
$51F_3$	0	2	1	6	.
$949F_2$	2	2	5	3	.
$961F_1$	1	3	8	1	.
$37F_3$	4	1	2	7	8
$43F_1$	3	2	3	6	.
$48F_2$	4	2	6	3	.
$48F_3$	4	2	2	4	.
$949F_2$	1	2	4	1	.
$961F_1$	0	0	2	1	.
$961F_5$	2	2	4	1	.
$37F_3$	1	1	1	2	X
$43F_1$	2	0	1	2	.
$48F_2$	2	0	1	2	.
$48F_3$	1	1	1	2	.
$961F_1$	0	0	2	1	.

* Classes:
+ + sera reacting with antisera to erythrocytes and to serum.
+ 0 sera reacting with antisera to serum but not with antisera to erythrocytes.
0 + sera not reacting with antisera to serum but does react with antisera to erythrocytes.
0 0 sera not reacting with either antiserum.
Numbers in parenthesis refer to doubtful reactions.

and the F_1 parent; however reactivity was still present for the sera of pigeons. The antipigeon sera are therefore complex but each recognized only a common substance among the backcross progeny.

Serum antigens of bison and cattle

The unique population of hybrids and backcross hybrids of bison and cattle at the Range Dominion Experiment Station, Manyberries, Canada made it possible to test for segregation of serum antigens in successive generations following the species cross. Extensive absorption of five antibison sera with the pooled normal sera of cattle yielded one antiserum which reacted specifically with bison serum.

TABLE 6

Flocculation tests of specific antipigeon sera with the sera of pigeon-ringdove hybrids and backcross hybrids

Group	Sire	Dam	Progeny	Antiserum designation			
				$42F_4$	$43F_4$	$961F_6$	$965F_3$
F_1	Unknown		G587Y	+	—	+	+
			$G587D_2$	+	—	+	+
			$G587F_2$	—	—	+	+
			$G587F_3$	—	—	+	+
			$G587\alpha_3$	+	—	+	+
			PD_1	+	—	+	+
			PD_3	—	—	+	—
			PD_5	—	—	0	—
			JTPD	+	+	—	0
1st backcross	JTPD(F_1)	Ringdove	$JTPDD_2$	+	+	+	0
			$JTPDD_3$	+	+	+	0
			$JTPDD_4$	+	0	+	0
			$JTPDD_5$	—	—	—	+
			$JTPDD_6$	+	+	0	0
			$JTPDD_8$	—	—	0	0
Pooled ringdove sera				0	0	0	0
Pooled pigeon sera				+	+	+	+

The remaining four sera were of such low titer following absorption that they could not be used to type the backcross sera. Among the F_1 hybrids the sera of 23 reacted with the antibison specific sera and that of one failed to react. Among the first backcross, 28 sera reacted and seven did not; in the second backcross population 13 reacted and six did not. These results are indicative of a successive replacement with genes from the recurrent cattle parent of genes contributed by the bison. Typing results with families in which the hybrid parent possessed reactive sera and possessed at least one nonreactive progeny were treated in the same way as the data from the *humilis* × ringdove families. The test for segregation among the hybrids as presented in Table 3 provides evidence for the hypothesis that segregation of serum antigens has occurred among the backcross progeny in accordance with a unifactorial mode of inheritance. The presence of large numbers of progeny in each generation with reactive sera indicates the presence of more than one antigenic difference which differentiates bison from cattle sera.

DISCUSSION

The results obtained in this study indicate that the antigenic substances of the serum segregate in backcrosses in accordance with a Mendelian mode of inheritance. Hybridizing species possess serum antigens in common but in addition contain species specific antigens. This confirms and extends the results already obtained by CUMLEY and IRWIN (1942, 1952) and CUMLEY, IRWIN and COLE (1941, 1943).

If there are species specific antigens, it is expected that all members of the species will possess them and that species hybrids would likewise contain the substance in their sera. It is also possible that some antigens may be restricted to a given species even though these substances are not found in all members of that species. It is shown in Table 3 that some F_1 hybrids possessed sera which did not result in flocculation with one or more absorbed antisera. The failure to react does not appear to be random with respect to the hybrids involved but the data are meagre with regard to this question. There were 56 mixtures of plasma or serum and antiserum, of these ten did not result in flocculation. Three hybrids together accounted for seven of these and the remaining three were distributed among the other hybrid sera.

The occurence of F_1 progeny whose sera are not reactive with the different antisera suggests segregation of some antigenic substances existing within the *humilis* parental group. An alternative is that quantitative differences are present among the hybrids with respect to the amount of antigenic material they possess. Some evidence which bears on this proposal was obtained from measurements of the total serum protein of hybrids using a refractometer. The mean percent protein of the 38 hybrids and backcross hybrids determined in this way was 4.07 ± 1.4 percent. There was no obvious association between failure to react and low serum protein concentration since all F_1 sera failing to react were either average or above average in concentration. This indicates that total serum protein content is not a consideration. In order to determine whether the concentration of a specific antigen is involved, isolation of the specific antigen which differentiates the *humilis* from the ringdove sera will be necessary. A specific antiserum which promotes the optimum production of precipitate could be used to test for heterogeneity of antigen concentration among the F_1 hybrids and the parental species and to compare their respective antigen titers. Lacking such a serum, nitrogen determinations of the precipitates of F_1 hybrid sera at constant antibody concentrations may provide evidence for heterogeneity.

An analysis of the sera of members of the parental species would also show whether some individuals completely lack the antigen, which would suggest heterozygosity. Further evidence would come from intraspecific progeny tests of individuals which produce hybrids with nonreactive sera. Intraspecific heterogeneity may also result from the presence of inhibitors. Inhibitors in the serum could be detected by testing whether the serum in question inhibits a known reactive serum. An hypothesis of interallelic interaction resulting in inhibition at a much earlier stage than the formation of serum antigens would require progeny tests and analyses of embryonic materials for inhibition.

The serum antigens which distinguish one species from another are in all likelihood associated with the serum proteins although there is no direct evidence for this postulate. In the event that such is found to be the case the differences will most likely be manifested in the serum globulins. When free electrophoresis of the sera of cattle, bison and their hybrids was conducted, differences were observed in the number of globulin components which distinguish bison from cattle and

the hybrid from each parent. These differences are most clearly seen in the electrophoretic patterns of Figure 1 in which the diagonal slit angle used was 55° for bison and cattle sera and was 65° for the hybrid sera. The complete patterns at a lower angle are shown in Figure 2. The details concerning these patterns are summarized in Table 7. Eight peaks could be distinguished in the globulin pattern of pooled cattle sera whereas only six were seen in the electrophoretic pattern of pooled bison sera. Some of the hybrid serum patterns contained eight distinguishable globulin peaks. The mobilities of two of the peaks do not coincide with either of the parental sera, moving more slowly than the gamma globulin components of cattle or bison sera. The sera of three of the hybrids as well as bison serum also possessed serum components moving more slowly than albumin, the so-called prealbumins. The number of these components in the hybrids is quite variable, one hybrid sera possessed three of these components, another possessed two and a third possessed one as did the serum of bison. The amount of these components is extremely small as judged by the magnitude of the peaks, and therefore slight

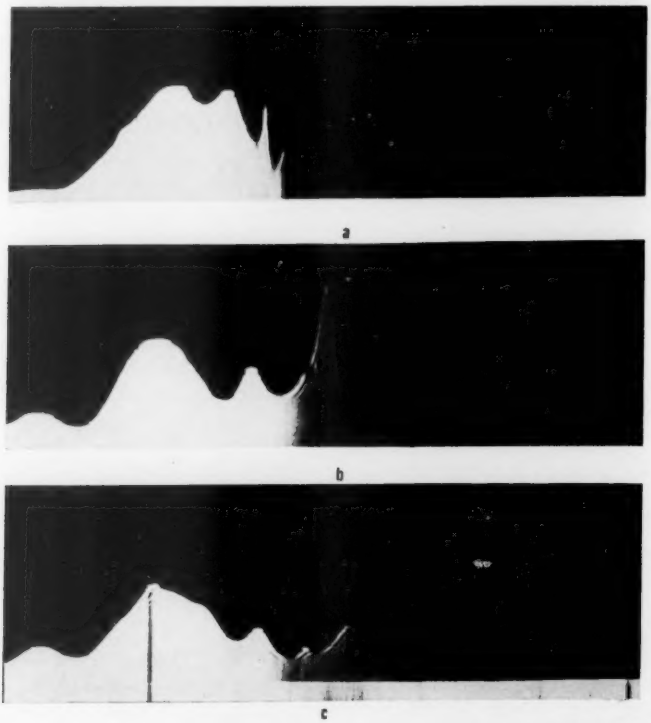

FIGURE 1.—Electrophoretic patterns of sera, descending boundary. a. pooled cattle sera. b. Pooled bison sera. c. Serum of bison-cattle hybrids.

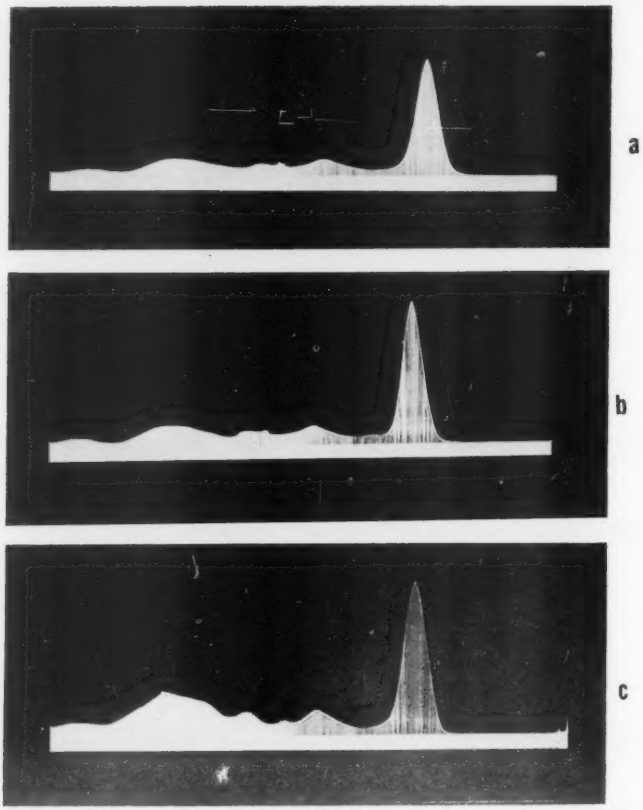

FIGURE 2.—Electrophoretic patterns of sera, descending boundary. a. Pooled cattle sera. b. Pooled bison sera. c. Serum of bison-cattle hybrid.

disturbances in the preparation of the serum for electrophoresis will result in the absence of these peaks from the electrophoretic pattern. In Figure 3 are presented the starch gel patterns of the sera of cattle, bison and the hybrid. The pattern of the hybrid possesses more distinguishable components than the sera of either parental species. This was not evident from the results of free electrophoresis and may be accounted for by the superior resolving power of the starch gel procedure. The electrophoretic patterns of the hybrids suggest that the serum contains all of the proteins present in each parental serum as well as additional components only present in the hybrid serum.

The electrophoretic patterns of *humilis*-ringdove sera and pigeon-ringdove sera are shown in Figures 4 and 5. These are tracings of the electrophoretic patterns which were made because the photographs contained components too faint

TABLE 7

Mobilities of electrophoretic components of species and species hybrids*

Sera	Prealbumins			Albumin	Globulins										Concentration (%)
Pool from 70 cows	...	...	...	6.98	6.07	5.53	4.92	4.38	3.98	...	3.38	2.50	1.61	...	...	2.66
Pool from 17 female bison	...	...	...	6.51	...	5.56	...	4.42	3.78	3.46	3.25	...	1.61	...	...	2.31
Pool from 93 male bison	...	8.4	...	6.90	6.00	...	4.80	...	3.60	3.40	...	2.05	...	...	...	2.35
Pool from 93 male bison	...	...	...	7.00	6.00	...	4.80	4.15	3.80	3.40	...	1.90	...	...	...	1.39
Bison cattle hybrid no. 2	...	...	...	6.35	5.57	...	4.40	...	3.75	...	2.92	2.28	1.58	...	...	1.91
Bison cattle hybrid no. 21	...	...	...	6.70	...	...	4.68	4.23	3.92	...	3.22	2.49	1.91	1.45	1.03	2.13
Bison cattle hybrid no. 50	8.86	8.70	7.88	6.54	5.78	...	4.67	...	3.86	...	3.02	2.19	1.73	1.34	0.90	2.38
Bison cattle hybrid no. 65	...	...	7.52	6.59	...	...	4.58	...	3.90	...	3.13	2.42	1.84	1.37	0.80	2.86
Pool from 100 ringdoves†	...	...	...	7.28	5.89	...	4.22	...	...	...	3.09	2.09	...	1.54	...	1.07
Pool from 30 *humilis*†	...	...	7.90	6.4	...	...	4.92	4.50	...	3.41	...	2.40	1.93	...	...	1.11
Pool from 10 *humilis* × ringdove hybrids†	...	...	7.75	6.25	5.46	...	4.71	...	...	3.38	2.86	2.23	1.74	...	...	1.09
Pool from 23 pigeons	...	...	...	6.21	...	5.32	4.90	...	3.83	...	2.81	2.34	1.72	...	...	1.50
Pool of two pigeon × ringdove hybrids†	...	...	7.26	5.89	...	...	4.64	...	...	3.35	3.16	2.23	1.57	...	...	1.19

* Mobilities × 10^{-5}.
† Birds starved for 24 hours prior to bleeding to diminish the lipid level in the serum.

to be reproduced. The patterns suggest that the hybrid possesses all of the serum protein found in the sera of each of the parental species.

The albumin-globulin ratios of the species sera as well as the hybrids are presented in Table 8. The variation in the albumin-globulin ratios among the hybrids presenting values: (a) close to one of the parents as in the case of the bison-cattle hybrid, (b) intermediate in value as in the case of the *humilis*-ringdove hybrid

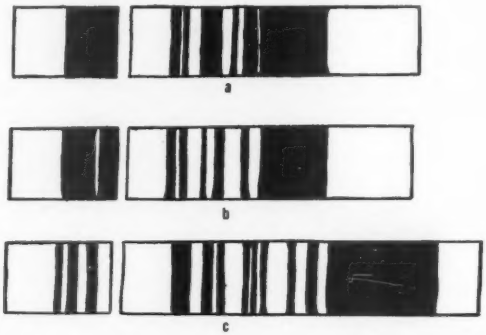

FIGURE 3.—Starch gel electrophoresis patterns of sera of bison, cattle and their hybrid. a. Pooled sera of cattle. b. Pooled sera of bison. c. Serum of bison-cattle hybrid No. 68.

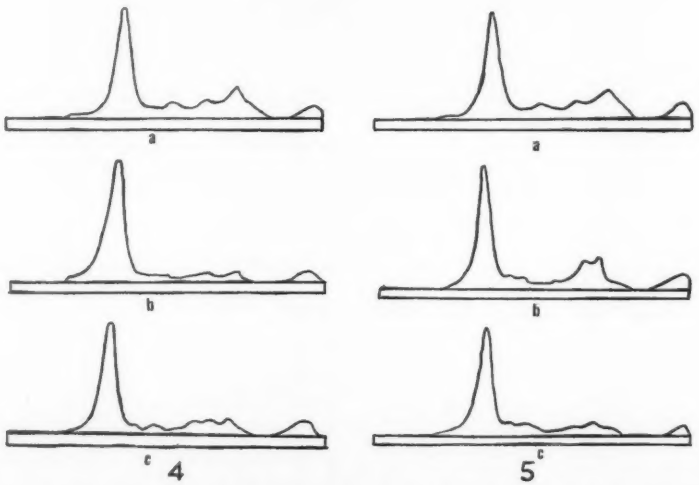

FIGURE 4.—Electrophoretic patterns of sera, ascending boundary. a. Pooled ringdove sera. b. Pooled *humilis* sera. c. Pooled *humilis* × ringdove sera.

FIGURE 5.—Electrophoretic patterns of sera, ascending boundary. a. Pooled ringdove sera. b. Pooled pigeon sera. c. Pooled sera of pigeon-ringdove hybrids.

TABLE 8

Relative concentrations of serum proteins in the sera of species and species hybrids

Species and number of animals in serum pool (in parentheses)	Relative concentration of albumin in percent	Relative concentration of globulin in percent	Ratio of albumin to globulin	Protein concentration in percent
Cattle, female (70) (mean of two determinations)	39.72	60.78	.65	2.66
Bison, female (17)	55.80	44.20	1.26	2.31
Bison, male (93)	52.38	47.62	1.10	1.39
Bison-cattle hybrids (mean of 4 hybrids of both sexes)	39.59±4.7	60.41	.66	2.37
Ringdove (100)	60.43	39.57	1.53	1.07
humilis (30)	83.30	16.70	4.99	1.11
humilis × ringdove hybrids (10)	63.79	36.21	1.76	1.09
Pigeon (23)	61.19	38.81	1.45	1.50
Pigeon × ringdove (2)	68.84	31.16	2.28	1.19

and (c) greater than either parental value as it is for the pigeon-ringdove hybrid is analogous to the genetic instances of dominance, genes lacking dominance and the presence of overdominance. Whether one should expect all of these different gene mechanisms to operate on seemingly the same kind of synthesis is a subject for much future experimentation.

The data on electrophoresis indicate that the major genetic differences between species lies in their globulins since the hybrids differ from the parental species mainly in this serum fraction. Both THOMPSON *et al.* (1954) and SMITHIES and WALKER (1956) have found a genetic basis for the differences within species among the globulins they studied. Species may differ in the other serum components but it seems likely that an intensive genetic study of the globulins will be more rewarding at this time. This is especially appropriate for experiments in which purified serum constituents are the subject of study.

SUMMARY

Specific antisera have been prepared against the serum antigens of *humilis*, pigeon and bison. With these, segregation was observed among the backcross progeny of *humilis*-ringdove, pigeon-ringdove and bison-cattle crosses. An hypothesis of single gene inheritance for each serum antigen is proposed to account for the results obtained.

ACKNOWLEDGMENT

The author is pleased to acknowledge the helpful suggestions and criticisms in the preparation of this paper from PROFESSOR M. R. IRWIN.

The author was fortunate in having access to unique populations from which blood samples were obtained. He is indebted to PROFESSOR M. R. IRWIN, University of Wisconsin for the use of dove and pigeon hybrids and backcross hybrids; to

MR. JIM TELFORD, Madison, Wisconsin for use of hybrids and backcross hybrids of pigeon × ringdove; and to DR. H. PETERS and DR. J. MCARTHUR, supervisors of the Dominion Agricultural Experiment Station, Manyberries, Alberta, Canada for assistance in obtaining blood samples from the bison × cattle hybrids and backcross hybrids.

LITERATURE CITED

ALBERTY, R. A., 1948 Introduction to electrophoresis. I. Methods and calculations. II. Analysis and theory. J. Chem. Educ. **25**: 426–433; 619–625.

CUMLEY, R. W., and M. R. IRWIN, 1940 Differentiation of sera of two species of doves and their hybrid. Proc. Soc. Exptl. Biol. Med. **44**: 353–355.

1942 Immunogenetic studies of species: segregation of serum components in backcross individuals. Genetics **27**: 177–192.

1943 Individual specificity of human serum. J. Immunol. **46** (2): 63–70.

1952 An immunogenetic study of species-specific antigens in the serum of species of Streptopelia. Genetics **37** (4): 396–412.

CUMLEY, R. W., M. R. IRWIN, and L. J. COLE, 1941 Genic effects on serum proteins. Proc. Natl. Acad. Sci. U.S. **27**: 565–570.

1943 Genic control of species-specific antigens of serum. J. Immunol. **47**: 35–51.

DRAY, SHELDON, and GLENDOWLYN O. YOUNG, 1958 Differences in the antigenic components of sera of individual rabbits as shown by induced isoprecipitins. J. Immunol. **81** (2): 142–149.

GALATIUS-JENSEN, F., 1957 Further investigations of the genetic mechanism of haptoglobins. Acta Genet. et Statist. Med. **7**: 549–564.

GITLIN, DAVID, WALTER H. HITZIG, and CHARLES A. JANEWAY, 1956 Multiple serum protein deficiencies in congenital and acquired agammaglobulinema. J. Clin. Invest. **35** (11): 1199–1204.

IRWIN, M. R., 1939 A genetic analysis of species differences in Columbidae. Genetics **24**: 709–721.

IRWIN, M. R., and L. J. COLE, 1940 Further studies of the interrelationships of cellular characters in Columbidae. Genetics **25**: 326–336.

LANDSTEINER, K., 1948 *The Specificity of Serological Reactions*. Rev. Ed. Harvard University Press. Cambridge, Mass.

LANDSTEINER, K., and J. VAN DER SCHEER, 1924 Serological examination of a species hybrid. I. On the inheritance of species specific qualities. J. Immunol. **9**: 213–219.

1940 On cross reactions of egg albumin sera. J. Exptl. Med. **71**: 445–454.

NUTTALL, G. H. F., 1904 *Blood Immunity and Blood Relationship*. Cambridge University Press. New York.

SASAKI, L., 1935 Precipitation test for a hybrid between Japanese long-tailed fowl and White Leghorn. Z. Zücht. Reihe B. Z. Tierzucht. Zuchtungsbiol. **32**: 95–108.

1937 Precipitation test for a generic hybrid between guinea hen and Leghorn cock. Z. Zücht. Reihe B. Z. Tierzucht Zuchtungsbiol. **38**: 361–365.

SMITHIES, O., 1955 Grouped variations in the occurrence of new protein components in normal human serum. Nature **175**: 307.

SMITHIES, O., and M. F. WALKER, 1956 Notation for serum protein groups and the genes controlling their inheritance. Nature **178**: 694–695.

STERN, CURT, 1949 *Principles of Human Genetics*. W. H. Freeman and Co. San Francisco.

THOMPSON, SARANE, JOSEPH E. FOSTER, JOHN W. GOWEN, and OSCAR E. TAUBER, 1954 Hereditary differences in serum proteins of normal mice. Proc. Soc. Exptl. Biol. Med. **87**: 315–317.

TEMPERATURE DEPENDENCE OF THE SPONTANEOUS MUTATION RATE TO RESPIRATION DEFICIENCY IN SACCHAROMYCES[1]

M. OGUR, S. OGUR AND R. ST. JOHN

Microbiology Department and Biological Research Laboratory, Southern Illinois University, Carbondale, Illinois

Received July 22, 1959

THE frequency of spontaneous genic mutations with increasing temperature has been reported to increase (MULLER 1928; PLOUGH 1941), decrease BEALE and FABERGÉ 1941; RHOADES 1941) or remain constant (DEMEREC 1932; WITKIN 1953) in different organisms. Temperature effects on the loss of various extrachromosomal hereditary determinants have also been reported (PREER 1948; PRINGSHEIM and PRINGSHEIM 1952). In the cytoplasmic mutation to respiration deficiency in Saccharomyces, YCAS (1956) observed higher mutant frequencies in *S. cerevisiae* strain grown at 40°C than in cultures grown at 30°C. GUTZ (1957) has reported similar observations with a *S. carlsbergensis* strain grown at 28°C compared to cultures grown at 7°C. Since the growth media employed in these studies allowed both respiration deficient (*aer*) and respiration sufficient (*AER*) cells to multiply, it was not determined whether the increased growth temperature induced or selected for the mutants. SHERMAN (1958) has offered evidence that the increased frequency of *aer* mutants in *S. cerevisiae* strains grown at 38°C (compared to 30°C) arose by induction rather than selection.

The current report deals with a study of the temperature dependence of the cytoplasmic mutation rate to respiration deficiency in five Saccharomyces strains at six growth temperatures between 15°C and 38°C.

MATERIALS AND METHODS

The yeasts employed were from the Carbondale collection of inbred Saccharomyces strains. They were chosen for study because their unclustered growth habit was appropriate to population analysis.

The glucose nutrient agar employed routinely was yeast extract-peptone nutrient agar containing one percent glucose. Stock cultures were stored on this medium under sterile mineral oil at 4°C. The selective agar differed by the substitution of sodium lactate (approximately one percent end conc., pH 5.5) for glucose. The selective liquid growth medium differed from the latter by omission of the agar. For details of media composition see OGUR and ST. JOHN (1956).

Inocula from storage slants were grown 24 hours on glucose nutrient agar. Cells were harvested and transferred to 250 ml Erlenmeyer flasks containing

[1] This work was aided by grants from the American Cancer Society and the National Science Foundation.

50 ml of the selective liquid medium at an initial density of 10^3 cells per ml. Flasks were shaken in Dubnoff metabolic incubators at controlled temperatures. Cultures were maintained in exponential phase by repeated transfer. Growth rates were estimated from hemacytometer counts. After at least ten generations had elapsed, appropriately diluted aliquots were spread on 50 glucose nutrient agar plates to yield approximately 200 colonies per plate. After four days of growth at room temperature, colonies were overlaid with agar containing 2,3,5 triphenyl tetrazolium chloride (TTC) yielding red (*AER*), white (*aer*) and some variegated (mixed) colonies (OGUR, ST. JOHN and NAGAI 1957). Mutant frequencies were generally estimated as the frequency of whole white colonies after TTC overlay. With a highly mutable strain (14940) at elevated temperatures, the mutant frequency estimate was affected by postplating growth in the presence of glucose. Mutant frequencies at 35°C and 38°C for this strain were therefore estimated by differential plate counts on glucose and lactate agar (OGUR and ST. JOHN 1956). These estimates were somewhat lower than those obtained by the TTC overlay procedure.

The selective medium allows *AER* cells to grow and produce either normal or mutant offspring, is totally selective against established *aer* mutants, but allows up to five phenomic lag divisions for newly formed *aer* mutants (OGUR, ST. JOHN, and OGUR 1959). We have previously shown that where mutant growth and back mutation are excluded, continued growth of a population of normal cells will produce an equilibrium frequency of mutants which is numerically equal to the mutation rate (OGUR, ST. JOHN, OGUR, and MARK 1959).

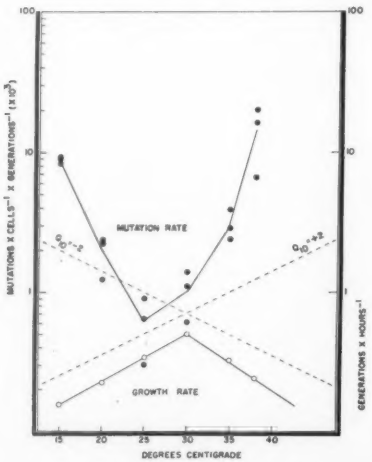

FIGURE 1.—Temperature dependence of growth in mutation to respiration deficiency —strain 15220 × 8256.

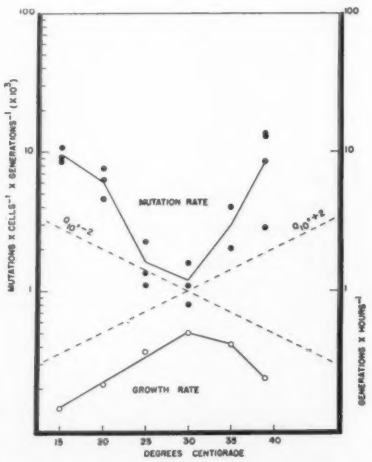

FIGURE 2.—Temperature dependence of growth and mutation to respiration deficiency strain 14716 × 8256.

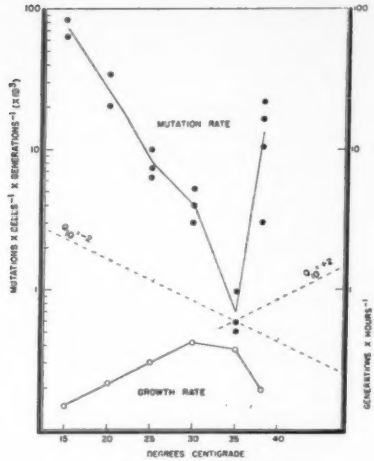

FIGURE 3.—Temperature dependence of growth and mutation to respiration deficiency —strain 8324 × 8282.

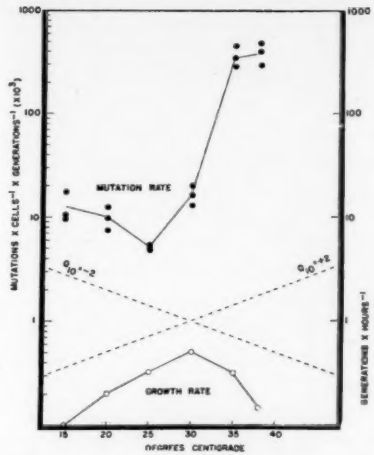

FIGURE 4.—Temperature dependence of growth and mutation to respiration deficiency —strain 14940.

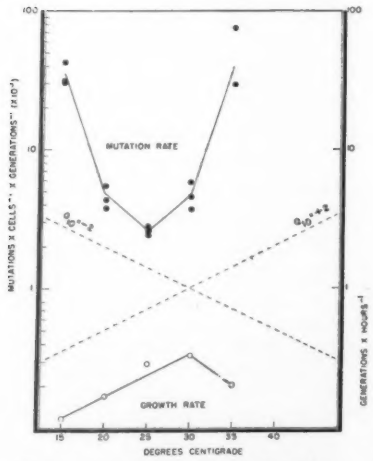

FIGURE 5.—Temperature dependence of growth and mutation to respiration deficiency —strain 13516.

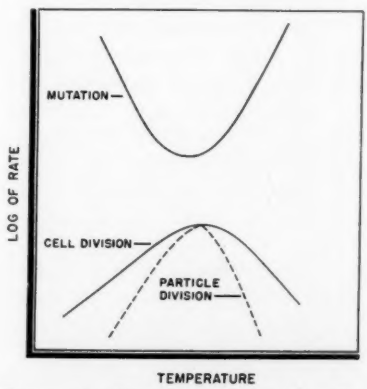

FIGURE 6.—Generalized curves for the temperature dependence of mutation to respiration deficiency and the division of yeast cells and hereditary particles.

RESULTS

Figures 1–5 summarize the growth and mutation rates for the five strains studied during steady-state growth in exponential phase in the selective medium. The dotted line curves represent reference slopes for Q_{10}'s equal to 2. The growth rate curves are based on points representing mean values calculated from 3–10 independent estimates at each temperature. It is apparent that these strains exhibit conventional temperature dependence of their growth rates in the selective medium employed, with Q_{10}'s equal to or slightly greater than 2 in the lower temperature range, maximum growth rate at 30°C and diminishing growth rate at higher temperature. One strain (13516) failed to grow at 38°C; the other strains failed to grow at 40°C in the medium employed.

Three separate estimates of the mutation rate were generally made at each temperature and have been plotted as separate points. The curves obtained are qualitatively comparable in the sense that the mutation rate exhibits a negative dependence in the low temperature range, then passes through a minimum and exhibits a large positive temperature dependence in the higher temperature range. It is apparent that both high growth temperature as well as low growth temperature enhance the mutation rate. In control experiments with cells incubated at the same temperatures in the absence of growth, no induction of mutants was observed.

One strain (14940) exhibits a large positive temperature dependence (Q_{10} ca. 350) between 30°C and 35°C and another (8324 × 8282) a large negative temperature dependence (Q_{10} ca. -10) between 15°C and 35°C. Strain 14940 shows marked instability during growth at 35°C (mutation rate ca. 400×10^{-3}) at which temperature strain 8324 × 8282 exhibits a mutation rate comparable to several strains (ca. 20×10^{-3}). On the other hand, strain 8324 × 8282 shows considerable instability during growth at 15°C (mutation rate ca. 75×10^{-3}) at which temperature strain 14940 exhibits a mutation rate comparable to several other strains (ca. 12×10^{-3}). Instability appears therefore to be strain dependent and is exhibited in both high and low temperature ranges.

DISCUSSION

The requirement for growth in the induction of mutation to respiratory deficiency has been noted previously with various inducing agents or conditions (EPHRUSSI *et al.* 1949; SHERMAN 1958). The current study confirms and extends this to inducing effects of both suboptimal and supraoptimal growth temperatures. That these effects are due to induction rather than cell selection is inherent in the procedure employed. Since the growth medium is totally selective *against* mutant cells, selection *for* mutant cells is excluded. The low frequency of inviable cells also excludes the possibility that selective killing of normal cells could account for the increased mutant frequency. The requirement for growth and the mutagenic effect of both high and low temperatures also appears to exclude any single

interpretation of the data based upon the direct thermal inactivation of a temperature sensitive site.

The irreversible, vegetatively inheritable loss of respiratory competence in yeast has been interpreted as the loss or functional inactivation of a semiautonomous factor (particle, site, plasmid) required for the biosynthesis of a component of the respiratory system (EPHRUSSI and HOTTINGUER 1950; LEDERBERG 1952). This loss mutation has been termed cytoplasmic (extranuclear, extrachromosomal, nongenic, non-Mendelian) because it may not be transmitted through an outcross to normal, and because the normal phenotype may be reconstituted when the cytoplasmic mutant is crossed to the complementary genic mutant (CHEN, EPHRUSSI, and HOTTINGUER 1950).

The spontaneous cytoplasmic mutation rate may be viewed as the probability that a bud will fail to receive any of the inheritable particles. This may be expected to depend on the number and distribution of inheritable particles, which would depend in turn on the rate at which particles were reproduced compared to the rate at which the rest of the cell were reproduced. Balanced growth may be viewed as the result of certain growth conditions which maintain essentially the same reproductive rate of cells and particles. Unbalanced growth due to differences in the reproductive rates of some cellular organelles may lead either to death (COHEN and BARNER 1954) if the organelle is essential or to a loss mutation if it is semiautonomous and expendable. Reasoning from this widely held theoretical model of the cytoplasmic loss mutation and the data obtained, one can deduce the general shape of the curve of temperature dependence of the reproduction of the cytoplasmic particles as represented in Figure 6. The uppermost and middle curves are generalized representations of the mutation rate and growth rate curves observed in Figures 1–5. The dotted line curve is a generalized curve deduced for the temperature dependence of the reproductive rate of the hereditary particles. This must be a maximal curve whose maximum may or may not be coincident with the growth rate maximum and whose arms must have steeper slopes than the growth rate curve. The coincident region of the two maxima curves would represent the condition of balanced growth, where the probability of a new cell receiving no particles might be expected to be minimal (the mutation rate would be lowest). At higher or lower temperatures, divergent rates of cell and particle reproduction (unbalanced growth) might be expected to increase the probability of a new cell receiving no particles (the mutation rate would be increased). It thus becomes possible to interpret the minimum curve for the temperature dependence of the mutation rate as the resultant of two divergent maxima curves for the temperature dependence of cell and plasmid reproduction.

SUMMARY

The temperature dependence of the cytoplasmic mutation rate to respiration deficiency has been studied in five Saccharomyces strains over the range from 15°C to 38°C.

The unusual minima curves obtained may be interpreted as the result of unbalanced growth at both low and high temperatures leading to an increase in the probability of the irreversible loss of an organelle essential to the biosynthesis of the respiratory system.

LITERATURE CITED

BEALE, G. H., and A. C. FABERGÉ, 1941 Effect of temperature on the mutation rate of an unstable gene in *Portulaca grandiflora*. Nature **147**: 356–357.

CHIEN, S., B. EPHRUSSI, and H. HOTTINGUER, 1950 Nature genetique des mutants a deficience respiratoire de la souche B-II de la levure de boulangerie. Heredity **4**: 337–351.

COHEN, S. S., and H. D. BARNER, 1954 Studies on unbalanced growth in *Escherichia coli*. Proc. Natl. Acad. Sci. U. S. **40**: 885–893.

DEMEREC, M., 1932 Effect of temperature on the rate of change of the unstable miniature –3 gamma gene of *Drosophila virilis*. Proc. Natl. Acad. Sci. U. S. **18**: 430–434.

EPHRUSSI, B., H. HOTTINGUER, and A. CHIMENES, 1949 Action de l'acriflavine sur les levures 1. La mutation "petite colonie". Ann. Inst. Pasteur **76**: 351–367.

EPHRUSSI, B., and H. HOTTINGUER, 1950 Direct demonstration of the mutagenic action of euflavine on baker's yeast. Nature **166**: 956.

GUTZ, H., 1957 Zur entstehung von atmungsdefekten mutanten bei einem stamm von *Saccharomyces carlsbergensis*. Naturwissenschaften **44**: 545.

LEDERBERG, J., 1952 Cell genetics and hereditary symbiosis. Physiol. Rev. **32**: 403–430.

MULLER, H. J., 1928 The measurement of gene mutation rate in Drosophila, its high variability, and its dependence upon temperature. Genetics **13**: 279–357.

OGUR, M., and R. ST. JOHN, 1956 A differential and diagnostic plating method for population studies of respiration deficiency in yeast. J. Bacteriol. **72**: 500–504.

OGUR, M., R. ST. JOHN, and S. NAGAI, 1957 Tetrazolium overlay technique for population studies of respiration deficiency in yeast. Science **125**: 928–929.

OGUR, M., R. ST. JOHN, and S. OGUR, 1959 Direct experimental observation of cells in phenomic lag. Science **129**: 1612–1613.

OGUR, M., R. ST. JOHN, S. OGUR, and A. M. MARK, 1959 The direct estimation of mutation rate from mutant frequency under special conditions. Genetics **44**: 483–496.

PLOUGH, H. H., 1941 Spontaneous mutability in Drosophila. Cold Spring Harbor Symposia Quant. Biol. **9**: 127–137.

PREER, J. R., JR., 1948 A study of some properties of the cytoplasmic factor, "kappa", in *Paramecium aurelia*, variety 2. Genetics **33**: 349–404.

PRINGSHEIM, E. G., and O. PRINGSHEIM, 1952 Experimental elimination of chromatophores and eye-spot in *Euglena gracilis*. New Phytologist **51**: 65–76.

RHOADES, M. M., 1941 Genetics of mutability in maize. Cold Spring Harbor Symposia Quant. Biol. **9**: 138–144.

SHERMAN, F., 1958 A study of the effects of elevated temperatures on the growth and inheritance of *Saccharomyces cerevisiae*. Thesis, Univ. of Calif. Berkeley. UCRL 8573.

WITKIN, E. M., 1953 Effects of temperature on spontaneous and induced mutations in *Escherichia coli*. Proc. Natl. Acad. Sci. U. S. **39**: 427–433.

YCAS, M., 1956 A hereditary cytochrome deficiency appearing in yeast grown at an elevated temperature. Exptl. Cell Research **10**: 746.

A STUDY OF HOMOLOGY BETWEEN A TERMINAL PORTION OF ZEA CHROMOSOME 2 AND A SEGMENT DERIVED FROM TRIPSACUM[1]

MARJORIE P. MAGUIRE

Genetics Foundation, University of Texas, Austin 12, Texas

Received August 10, 1959

DURING studies on cytogenetics of *Zea mays* hyperploid for chromosomes derived from *Tripsacum dactyloides* (MAGUIRE 1957), a 2N + 1 stock was isolated containing a pair of Zea-Tripsacum interchange chromosomes. One of these carried a terminal portion of Zea chromosome 2S attached to a segment of a Tripsacum chromosome bearing no apparent homology to any Zea chromosome. The reciprocal exchange contained the remainder of Zea chromosome 2 including its centromere and a portion of the Tripsacum chromosome (marked by a terminal knob) equal in length to the distal segment of Zea chromosome 2S for which it had been exchanged in an earlier backcross generation (Figure 1). A normal Zea chromosome 2 was present in this stock as well as two apparently normal mem-

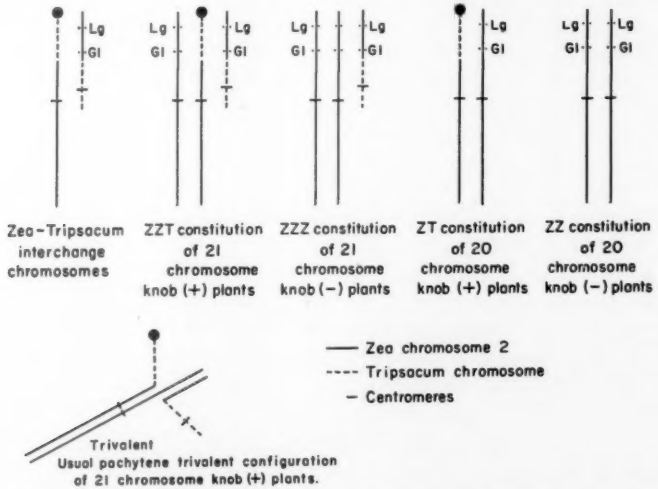
Lg
Gl
Zea-Tripsacum interchange chromosomes
ZZT constitution of 21 chromosome knob (+) plants
ZZZ constitution of 21 chromosome knob (−) plants
ZT constitution of 20 chromosome knob (+) plants
ZZ constitution of 20 chromosome knob (−) plants
Zea chromosome 2
Tripsacum chromosome
Centromeres
Trivalent
Usual pachytene trivalent configuration of 21 chromosome knob (+) plants.

FIGURE 1.—Diagrams showing composition of Zea-Tripsacum exchange chromosomes, the various combinations in which they occur and the typical pachytene trivalent configuration of 2N + 1 knob (+) plants.

[1] This work was supported by a grant from the Rockefeller Foundation and by National Science Foundation Grant number G7068.

bers of each of the other Zea chromosomes. In 2N + 1 plants pachytene trivalents (as illustrated in Figure 1) were found with high frequency (about 90 percent). In disomic plants the ZT exchange chromosome 2 was commonly paired throughout at pachytene with the normal chromosome 2 although pairing failure, usually terminal, occurred with varying frequency and extent. Genetic data indicated the presence of *Lg* and *Gl* genes in the Tripsacum segment.

With the purpose of finding the physical and genetic length of the Tripsacum segment in the ZT exchange chromosome and estimating the degree of its homology to the corresponding portion of Zea chromosome 2, the stock was crossed to a chromosome 2 "good pachytene" genetic tester carrying recessive lg_1 (liguleless leaf-1), gl_2 (glossy seedling-2), and v_4 (virescent seedling-4) alleles. For pachytene studies microsporocyte samples were fixed in alcohol-acetic 3:1 mixture and refrigerated until examined in acetocarmine smears.

RESULTS AND DISCUSSION

Genetic studies: To test recombination between the Tripsacum and Zea segments 20 chromosome plants with a terminal knob on chromosome 2 were selected from the T_1 progeny and pollinated by the chromosome 2 tester. These plants were assumed to be of ZT constitution (as illustrated in Figure 1) since in the 2N + 1 female parent plants trivalents were formed with a frequency of about 90 percent and their usual configuration was such that there was no opportunity for crossing over between Zea and Tripsacum segments. In the T_2 generation the recessives segregated in normal Mendelian fashion (Figure 2), and the *Lg* and *Gl* genes from Tripsacum appeared fully capable of dominating the recessive lg_1 and gl_2 contributions from Zea. There was only 0.2 percent recombination, however, between the Tripsacum and Zea segments in this region although there are 19 crossover units between lg_1 and gl_2 on the corn chromosome 2 map (Figure 3). Possible explanations for this recombination deficiency are that the degree of homology or synaptic union was inadequate for crossing over to occur with normal frequency, or that a gene acting as a crossover suppressor in this genotype was present. If a suppressor was present, its activity may have been directed at the region heterozygous for the Tripsacum segment which included *Lg-Gl* since Gl_2-V_4 recombination appeared more nearly normal. It would be difficult to distinguish the effect of a suppressor gene active over a limited chromosome portion from inhibition of crossing over caused by inadequate homology or synapsis, but it is interesting that in 2N + 1 ZZT plants most of the trivalents which were formed persisted until anaphase I. Therefore, if it is assumed that it is terminalized crossover chiasmata which hold them together until this time, crossing over occurred with an extremely high frequency between two corresponding Zea segments in this region in the presence of the Tripsacum segment.

Studies of pachytene pairing failure: To examine the degree of homology and synaptic union between the corresponding Zea and Tripsacum segments studies were undertaken of the frequency and extent of their pachytene pairing failure

FIGURE 2—ZT Segregation:

Culture No.	Gene	Observed	Expected	χ^2
14	Lg_1	156	162.5	.52
	lg_1	169	162.5	
15	Lg_1	89	86.5	1.44
	lg_1	84	86.5	
16	Lg_1	188	174.0	2.26
	lg_1	160	174.0	
Total	Lg_1	433	423.0	.48
	lg_1	413	423.0	
14	Gl_2	159	166.5	.68
	gl_2	174	166.5	
15	Gl_2	89	86.5	1.44
	gl_2	84	86.5	
16	Gl_2	189	174.0	2.8
	gl_2	159	174.0	
Total	Gl_2	437	427.0	.46
	gl_2	417	427.0	
14	V_4	158	166.0	.78
	v_4	174	166.0	
15	V_4	84	76.0	1.68
	v_4	68	76.0	
Total	V_4	242	242.0	0
	v_4	242	242.0	

FIGURE 2.—Genetic segregation data from progeny of ZT plants pollinated by a tester stock homozygous for lg_1, gl_2 and v_4. The exchange chromosome 2 of the female parents carried dominant *Lg* and *Gl* alleles in the Tripsacum segment and V_4 in the Zea segment, while the normal chromosome 2 of these plants carried lg_1, gl_2 and v_4.

in microsporocytes of ZT plants. Surprisingly, it was found that similar pairing failures occurred in terminal regions of all the chromosomes of the complement and occasionally interstitially as well. Some of these pairing failures are illustrated in Figure 4. In rare cases entire chromosomes were found to be univalent. It could not be immediately determined whether this pairing failure represents synaptic failure or precocious opening of parts originally paired or some mixture of both. Records were kept and measurements made where possible of all detectable pairing failures so that frequencies in the various chromosomes, extents and distributions could be compared. Slides were systematically scanned and camera lucida drawings were made wherever the clarity of configuration permitted and where chromosomes appeared not to be stretched and were flat. The same settings of camera lucida, microscope and drawing board were used for all drawings.

FIGURE 3—ZT Recombination

Culture No.	Region	Observed	Expected*	χ^2
14-parental	Lg_1–Gl_2	324	263	74
14-recomb.	Lg_1–Gl_2	1	62	
15-parental	Lg_1–Gl_2	173	141	41
15-recomb.	Lg_1–Gl_2	0	32	
16-parental	Lg_1–Gl_2	347	282	79
16-recomb.	Lg_1–Gl_2	1	66	
Total-parental	Lg_1–Gl_2	844	686	192
Total-recomb.	Lg_1–Gl_2	2	160	
14-parental	Gl_2–V_4	190	216	5.8
14-recomb.	Gl_2–V_4	142	116	
15-parental	Gl_2–V_4	79	99	11.6
15-recomb.	Gl_2–V_4	73	53	
Total-parental	Gl_2–V_4	269	315	18.4
Total-recomb.	Gl_2–V_4	215	169	

* Expected recombination in Lg_1–Gl_2 region = 0.19 (EMERSON, BEADLE, and FRASER 1935).

† Expected recombination Gl_2–V_4 region taken at 0.35 based on studies by RHOADES as reported in EMERSON, BEADLE, and FRASER 1935. Region includes 52 crossover units.

FIGURE 3.—Genetic recombination data from progeny of ZT plants pollinated by a tester stock homozygous for lg_1, gl_2 and v_4. The exchange chromosome 2 of the female parents carried dominant Lg and Gl alleles in the Tripsacum segment and V_4 in the Zea segment, while the normal chromosome 2 of these plants carried lg_1, gl_2 and v_4.

Magnification at drawing board level was measured directly with a stage micrometer. Linear measurements of chromosome drawings were made by stretching a flexible wire along the segment to be measured, straightening the wire and measuring it against a ruler. Such measurements were found to be reproducible to an accuracy of about 99 percent. Chromosomes were identified by means of knob markers, relative lengths and arm ratios. The results include records of pachytene pairing failure from microsporocytes of 39 disomic plants with a Zea-Tripsacum exchange chromosome 2 (referred to as knob (+) plants) and from 28 disomic plants with apparently all normal chromosomes (called knob (−) plants). All plants were progeny of 2N + 1 knob (+) plants which were thought to contain an intact (or nearly so) segment from Tripsacum for the reasons stated earlier.

Most of the pairing failures observed were terminal. Only about three percent of 8,410 cells analyzed showed intercalary pairing failure, while about 38 percent of these cells had at least one terminal failure.

The distribution of number of plants with various frequencies of pairing failures is illustrated in Figure 5. No plant was entirely free of failure. Microsporocytes from single anthers differed widely in their number and extent of pachy-

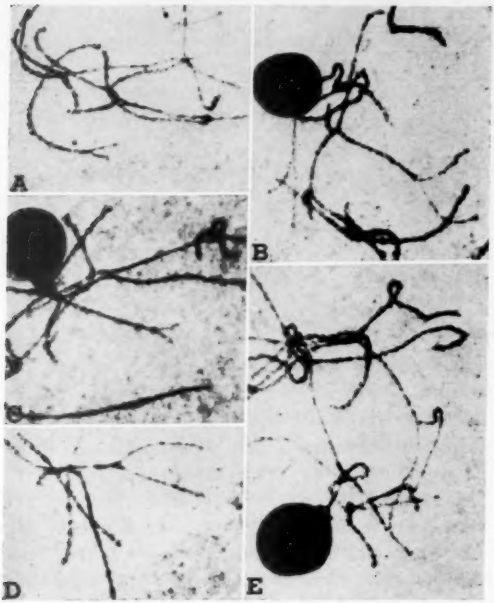

FIGURE 4.—Photomicrographs of cells showing chromosome terminal pairing failure at pachytene. Magnification is about 1000 ×. A.—short pairing failure in bivalent at lower center. B.—pairing failures in bivalents at lower left and lower right. C.—short pairing failure in bivalent just to the right of center. D.—pairing failure in bivalent at upper right. E.—long pairing failure in bivalent extending downward from center right. All of these occurred in the same anther of a chromosome 2 knob (—) plant.

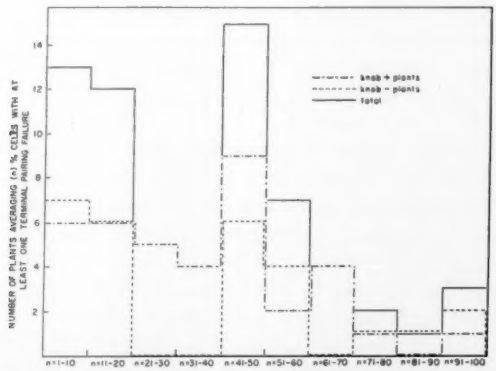

FIGURE 5.—The number of plants with various average frequencies of chromosome terminal pairing failure at pachytene.

tene pairing failures, and nearly all contained at least some cells in which all chromosomes were paired throughout. Where there was pairing it appeared normally intimate, and chromomeres were as well matched as usual. Further, while anthers of a flower differed by as much as 47 percent in frequency of cells with pairing failure, anthers from adjacent flowers centrally located on the same tassel branch, in the few plants where such comparisons were possible, differed by as much as 83 percent. Differences in pairing between plants might be related to differences in degrees of homology of parts involved, to differences in the amount of heterozygosity they contain, or to other genotypic effects, but they could as readily be attributed to sampling differences, since intraplant variation was so great. The differences in pairing failures observed here thus appear to be due, in large part at least, to response to differential environment of failure-susceptible genotype, apparently present in all plants, though the degree of susceptibility may have differed among them. A number of comparisons and correlations of failure distribution were studied and are described. Data from plants which undoubtedly differed genotypically were combined in a search for basic behavior patterns. These interplant genotypic differences may have been such as to constitute a source of error. To minimize error, where comparisons were drawn among chromosomes of the complement, only those cells were considered where values for all chromosomes were available, and for comparisons between cells, averages for the various plants were used, so that contributions were equally weighted. Nonparametric tests of correlation were applied to avoid the assumption of normal distribution (TATE and CLELLAND 1957).

Complete drawings and measurements were made of all chromosome material of 151 cells from 24 plants. In an attempt to find whether the extent of pairing failure was dependent on the stage of pachytene advancement observed, the total lengths of all chromosomes were added together in each of these 151 cells (and averaged for each of the 24 plants involved). Shorter total length is assumed generally to mark more advanced stages of pachytene in a given plant, although differences in pressure applied in making the squash preparations undoubtedly contributed to the variation. These measurements were plotted for each plant in Figure 6 against the average total extent of pairing failure per unit length of chromosome. A rank difference test of correlation gave an rd (rank difference correlation coefficient) of -0.508 for knob (+) plants, -0.670 for knob (−) plants (both significant at five percent level), and -0.847 (significant at one percent level) for the total of knob (+) and knob (−) plants. A similar comparison of total length and number of terminal failures (Figure 7) gave an rd of -0.879 (significant at one percent level) for knob (+) plants, -0.701 (significant at five percent level) for knob (−) plants, and -0.609 (significant at one percent level) for the total of knob (+) and knob (−) plants. Similarly the average total length of chromosome 2 as compared to its average extent (percent length) pairing failure from 125 cells of 16 knobs (+) plants gave an rd of -0.771, significant at one percent level. All of the 125 measurements are plotted in the scatter diagram of Figure 8. In knob (+) plants the average total length

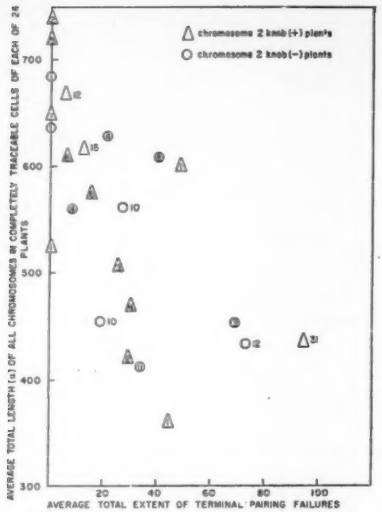

FIGURE 6.—Extent of chromosome terminal pairing failure at pachytene as a function of total chromosome length. For the total lengths, lengths of all the chromosomes were added together for each cell in which all chromosomes could be traced, and then all such totals were averaged for each of the 24 plants where completely traceable cells were found. In each cell the extent (percent length) of pairing failure was measured for each chromosome where failure occurred. These percentages were totaled for each cell and averaged for each of the 24 plants to give the average total extent of pairing failures. The number at each point indicates the number of cells for which values were averaged to give that point.

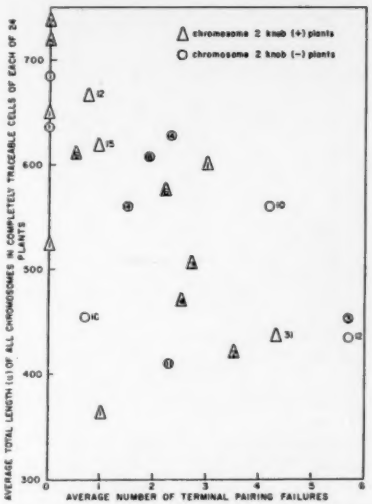

FIGURE 7.—Number of chromosome terminal pairing failures at pachytene as a function of chromosome length. For the total lengths, lengths of all the chromosomes were added together for each cell in which all chromosomes could be traced, and then all such totals were averaged for each of the 24 plants where completely traceable cells were found. The numbers of terminal pairing failures in completely traceable cells were also averaged for each of these plants. The number at each point indicates the number of cells for which values were averaged to give that point.

of chromosome 2 with terminal pairing failure of its short arm was 59 μ (125 measurements) as compared to 73 μ (23 measurements) for chromosome 2 with no pairing failure in its short arm. These results are evidence that many of the pairing failures observed, including those of the short arm of chromosome 2, represent dissociation beginning and increasing during pachytene.

In 63 of the 151 cells completely drawn it was possible to identify all the chromosomes. The numbers of terminal pairing failures for each chromosome of the corn complement in these 63 cells are plotted in Figure 9 against the relative lengths of the various chromosomes. A rank difference test of correlation of number of failures to relative chromosome length gave an rd value of +0.700 for knob (+) plants and +0.729 for knob (−) plants, both significant at five percent level,

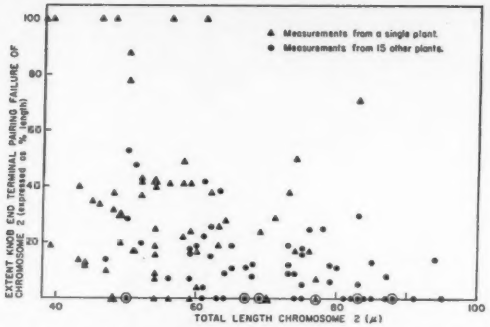
Measurements from a single plant.
Measurements from 15 other plants.
EXTENT KNOB END TERMINAL PAIRING FAILURE OF CHROMOSOME 2 (expressed as % length)
TOTAL LENGTH CHROMOSOME 2 (μ)

FIGURE 8.—Extent (percent length) of terminal pairing failure at pachytene of the short arm of chromosome 2 in knob (+) plants as a function of its total length. Measurements are from 125 cells of 16 plants; 62 of these measurements were from a single plant sample which contained an unusually large number of clear sporocytes at pachytene.

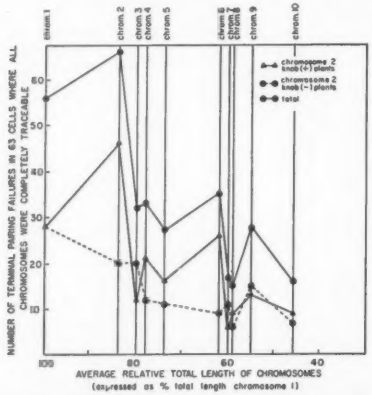

FIGURE 9.—Number of chromosome terminal pairing failures at pachytene as a function of relative chromosome length of the various chromosomes of the corn complement. The totals for each chromosome are from 63 cells of 16 plants where all chromosomes could be traced and identified. The relative chromosome lengths are standard values (expressed as percent length of chromosome 1).

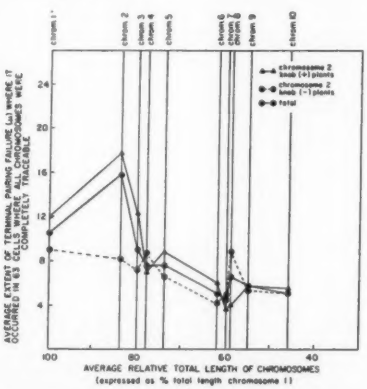

FIGURE 10.—Average extent of chromosome terminal pairing failure at pachytene as a function of relative chromosome length of the various chromosomes of the corn complement. The lengths of failures found in 63 cells (of 16 plants) where all chromosomes could be traced and identified were totaled and averaged for each of the ten chromosomes. The relative chromosome lengths are standard values (expressed as percent length of chromosome 1).

while their total gave an rd of +0.793 which is significant at the one percent level. The average extent of these failures for each chromosome is plotted against relative length in Figure 10 with an rd of +0.912 for knob (+) plants, an rd of +0.773 for knob (−) plants (if a single instance of complete pairing failure for

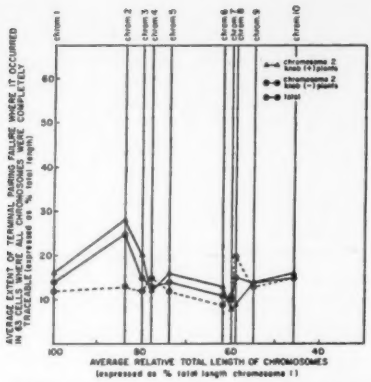
chrom 1
chrom 2
chrom 3
chrom 4
chrom 5
chrom 6
chrom 7
chrom 8
chrom 9
chrom 10
chromosome 2 knob (+) plants
chromosome 2 knob (−) plants
total
AVERAGE EXTENT OF TERMINAL PAIRING FAILURE WHERE IT OCCURRED IN 63 CELLS WHERE ALL CHROMOSOMES WERE COMPLETELY TRACEABLE (expressed as % total length)
AVERAGE RELATIVE TOTAL LENGTH OF CHROMOSOMES (expressed as % total length chromosome 1)

FIGURE 11.—Average extent (percent length) of chromosome terminal pairing failure at pachytene as a function of relative chromosome length of the various chromosomes of the corn complement. The percent length of chromosome involved was computed for each terminal pairing failure found in 63 cells (of 16 plants) where all chromosomes could be completely traced and identified. These percentages were then averaged for each of the ten chromosomes. The relative chromosome lengths are standard values (expressed as percent length of chromosome 1).

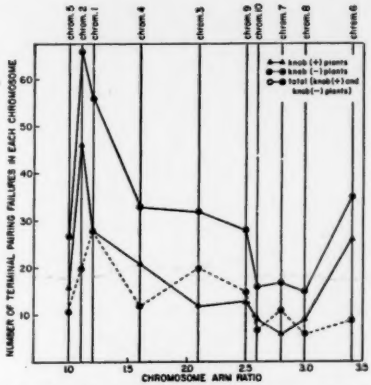

FIGURE 12.—Number of chromosome terminal pairing failures at pachytene as a function of chromosome arm ratio. The totals for each chromosome are from 63 cells (of 16 plants) where all chromosomes could be traced and identified. The arm ratios are standard values.

chromosome 8 is omitted from the calculations) and an rd of +0.852 for the total of knob (+) and knob (−) plants (with no omissions). All of these are significant at the one percent level. However, the average extent *per unit length* of these failures for each chromosome which is plotted in Figure 11 against relative chromosome length gave an rd of +0.491 (not significant at ten percent level) for knob (+) plants, and an rd of −0.091 for knob (−) plants and an rd of +0.127 for the total of knob (+) and knob (−) plants. Both of these latter rd's are not significant at the 20 percent level. The data suggest that the frequency of pairing failure tends to be greater in longer chromosomes, while the extent tends in general to be about constant per unit length of chromosome. In view of these facts it was tempting to speculate that longer chromosomes begin to dissociate earlier than shorter ones with the extension of the dissociation then proceeding at an approximately constant rate in all cases. A test of whether failures in later stages involve the shorter chromosomes more often than in earlier stages failed to support this hypothesis. The test involved a rank difference correlation of stage advancement (total length of all chromosomes) in each cell with an index for that cell composed of the ratio of the sum of the numbers representing those chromosomes of the complement which showed terminal pairing failure to the

total number of such chromosomes. The rd of this test was +0.060. It seems more likely therefore, that the proportional distribution of failures among the various chromosomes remains about constant throughout pachytene and that not only is the frequency of dissociation greater in longer chromosomes, but it also proceeds at a faster rate in them once begun. It is probably pointless to speculate at this time as to whether the differential behavior of longer chromosomes might be due to mechanical difficulties or to chemical effects, such as relative shortage of some key substance, or to some other reason. If the pairing failures represent precocious opening of synapsed chromosomes before the time for crossing over, they would prevent crossing over. If they represent such an opening after this time, their extent in each case might be conditioned by the position of the most distal chiasma. Genetic tests of possible crossover reduction and cytological studies of chiasma reduction at diakinesis are not practical because of the generally low and variable frequency of pairing failure. BARBER (1941) working with Uvularia explained a greater effect of temperature shock on chiasma reduction in long chromosome arms as due to a time limit of synapsis and greater freedom of movement for short arms. Such an explanation for a tendency of longer chromosomes to show more pairing failure here would be based on the questionable assumption that the failures observed represent initial failure of synapsis rather than precocious opening. REES (1958) found a retardation of meiotic prophase condensation in Scilla chromosomes to be more marked in the longer chromosomes of the complement. Since this retardation was found in a plant grown under unusual conditions, it was interpreted as another instance of greater vulnerability of normal processes in longer chromosomes to adverse factors.

To test whether pairing failure is related to centromere location, a correlation test was run of chromosome arm ratio to number of terminal failures (Figure 12). The rd for knob (+) plants was −0.525 (not significant at ten percent), for knob (−) plants, −0.642 (significant at five percent), and for the total of knob (+) and knob (−) plants, −0.447 (not significant at 20 percent). Some negative correlation of arm ratio to pairing failure might be expected, since, in corn, a rank difference test of correlation of arm ratio to relative length gives an rd of −0.636, significant at five percent level, and relative length appears to be somewhat positively correlated to failure frequency.

Fragmentary evidence suggests that the pairing failure observed is not related to the presence or absence of chromosome knobs. Aside from the Tripsacum segment only chromosome 9 may carry a terminal knob in this material. Of 60 cases of terminal failure in chromosome 9, 27 occurred at the knob end, and of these 11 were in plants homozygous for this knob.

As indicated in Figures 9 and 10 the values for pairing failure in chromosome 2S in knob (+) plants were markedly higher than for the other chromosomes and probably reflect a lesser degree of homology between corresponding Zea and Tripsacum segments than between two like Zea segments. The frequency of this failure in 3,814 cells was about seven percent, and in measurements from 125 cells its average extent was 22 percent of the length of chromosome 2. Since the

average frequency of terminal failure per chromosome end in 8,410 cells was about three percent, this frequency for chromosome 2S was about two and one half times the average value. When more measurements are available and these can be compared to measurements from trivalents in 2N + 1 plants, the relation of the length of the Tripsacum segment to the increase in pairing failure of chromosome 2S for which it appears to be responsible may be of interest. The occurrence of chromosome 2S pairing failure, however, appears to be weakly correlated with occurrence of pairing failure in the other chromosomes. An rd of +0.425 was found for a rank difference correlation of failure of cells with chromosome 2S failure compared to frac[illegible] of cells with [illegible] failure (not including chromosome 2) in 33 knob (+) plants (Figure 13). This correlation

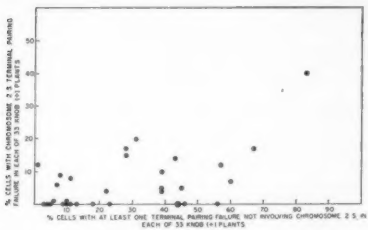
% CELLS WITH CHROMOSOME 2 S TERMINAL PAIRING FAILURE IN EACH OF 33 KNOB (+) PLANTS
% CELLS WITH AT LEAST ONE TERMINAL PAIRING FAILURE NOT INVOLVING CHROMOSOME 2 S IN EACH OF 33 KNOB (+) PLANTS

FIGURE 13.—Frequency of cells with terminal pachytene pairing failure of the short arm of chromosome 2 in each of 33 knob (+) plants as a function of frequency of terminal pairing failure involving other chromosomes. The data are observations of 2,459 cells distributed among the 33 plants. Data from six other knob (+) plants described in other parts of this report were collected in the early stages of the study and were not appropriately classified for this comparison.

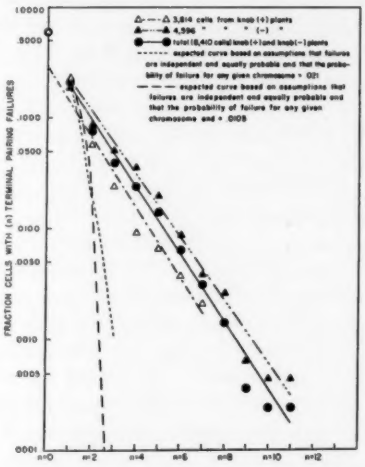

FIGURE 14.—Frequencies of pachytene cells with various numbers of chromosome terminal pairing failures.

was significant at five percent level, indicating a tendency for a high frequency of chromosome 2 failure to occur with a relatively high frequency of failure for the other chromosomes as well.

To determine the pattern of distribution of pairing failure frequency of cells with more than one failure, data were recorded from 8,410 cells as to the number of cells with 0, 1, 2, . . . 11 terminal failures (Figure 14). If it is assumed that events of pairing failure of various chromosomes are independent and equally probable, the probability that a cell will have N failures is given by the formula:

$$P = \frac{(N!)}{(R!)\ (N\text{-}R)!}\ (X)^R$$

where N is the total number of chromosomes or chromosome ends, R is the number of specified failures, and X is the probability that a given chromosome or

chromosome end will fail to be paired. In plotting the "expected" curves in Figure 14 it was assumed that the probability of failure for a specified chromosome is 0.021 (0.21/10) and for a specified chromosome end 0.0105 (0.21/20), where 0.21 is the observed frequency of cells with one terminal failure. The data obviously depart from random expectation, suggesting that failures are not independent, or are mutually dependent on other factors, such as conditions accompanying stage advancement or other environmental factors. Also the frequency of cells with exactly one failure in which that failure involved chromosome 2S in knob (+) plants was 0.13, indicating a bias in its favor. A similar bias was noted for chromosome 6S which represented 99 percent of single failures. Unfortunately the other chromosomes could be reliably identified for the most part only in the 63 cells where all chromosomes could be identified. The limited information available, however, suggests that a higher than average proportion of terminal failures also occurred in the long arm of chromosome 1 and in both arms of chromosome 9, at least in certain plants.

The fraction of cells with a chromosome showing terminal pairing failure of both ends was 0.0067 as opposed to the 0.0011 expected if the probability a specified end will fail to be paired is assumed to be 1/20 the frequency of a single terminal failure and failures are assumed to be independent and equally probable. (The probability that both ends of one chromosome will be unpaired is given by the formula NX^2 where X is the probability a given chromosome end will fail to be paired, and N is the total number of chromosome ends.)

Necessarily in most slides a number of cells contained chromosomes too poorly spread to be classified. Records were kept for each slide of the number of such unclassified cells. In a few cases less than five percent of cells were unclassified. The fraction of cells with at least one terminal failure from these slides was 1041/2569 or 41 percent as compared to 38 percent for all recorded data. Unfortunately the data amassed from these exceptional slides is inadequate for comparisons of other measurements with the grand totals, and so it must be borne in mind that some bias may be imposed by selection of classifiable cells. Cells with very large amounts of pairing failure are more difficult to analyze and therefore must be more frequently rejected than others.

Although careful comparisons to other stocks have not yet been possible, it is thought that the material under study is unusually high in general terminal pairing failure at pachytene. The amount of this pairing failure was sufficiently low, however, that it could have been overlooked in cursory examination, and diakinesis, metaphase and pollen appeared completely normal. Pairing failure is commonly observed by those who study corn pachytene slides in the satellite arm of chromosome 6 and less often in other usually unidentified regions. To study how much the material examined here departed from the normal, other stocks both homozygous and heterozygous are currently being examined. The abnormality was certainly less than that in "asynaptic" corn plants described by BEADLE (1933) and in "asynaptic" plants of other species which have been described by many workers. In these the pairing failures are directly attributable

to mutants and are often responsible for extreme irregularity at metaphase and anaphase I. Commonly only diakinesis, metaphase and anaphase I have been studied, and little or no attention has been directed at the earlier stages which are usually too complex for interpretation. The pairing failures observed here may be due to the replacement of terminal segments of corn chromosomes by segments from Tripsacum during the experimental generations. MANGELSDORF and REEVES (1939) reported a slight terminal dissociation in the long arm of chromosome 2 in a disomic plant of corn-Tripsacum backcross progeny. They interpreted this to mean that a short piece of a Tripsacum chromosome had been exchanged for a short segment of the end of chromosome 2. A comparatively long region in the present study known to be heterozygous for such a replacement showed a higher than average amount of failure, but it would be remarkable if all regions in which failure occurred were likewise heterozygous for a Tripsacum segment. In a few cases entire chromosomes were unpaired. It is possible that the presence of Tripsacum chromosome material or the absence of a corn segment predisposes a corn nucleus to general terminal dissociation at pachytene. In this case it would be necessary to assume that the knob (−) plants of this study (those without the Tripsacum segment on chromosome 2) also carried at least one and probably more Tripsacum segments (at present unidentified) since these plants were very similar in total pairing failure to knob (+) plants. "Asynapsis" has been reported in wheat plants carrying an extra rye chromosome by SADANAGA (1957), in oats lacking a pair of chromosomes by HUSKINS and HEARNE (1933) and in monosomic corn by MORGAN (1956). In these cases also the amount of the abnormality was greater than that observed here. It is interesting that MORGAN's plants also showed plasmodial sporocytes, a defect sometimes observed in the present study. Since homologous chromosomes may exhibit irregular pachytene pairing in slightly unbalanced complements, isolated observations of dissociation should not be assumed to indicate lack of homology of parts involved.

In most of the cases of "asynapsis" which have been reported a strong dependence on environmental factors is indicated, as it is here, because of great intraplant variation. It should be mentioned that noticeable amounts of terminal pairing failure were observed in this material in other generations grown under other conditions, although no detailed studies of it was conducted at those times.

In previous reports of "asynapsis" no analysis of pachytene behavior was included, so it cannot be said whether terminal as opposed to intercalary failure is a special peculiarity of this material.

If pairing failure at the knob end of chromosome 2 in megasporocytes of the three plants in which recombination was tested was consistently as high as in a few of the microsporocyte samples studied here, it alone could account for the rare recombination observed, assuming the chromosomes were unpaired at the appropriate time. However, the average frequency of pairing failures observed in this region (seven percent) was inadequate to prevent much crossing over. Studies of microsporocyte samples from the same plants tested and general comparisons of microsporocytes to megasporocytes may resolve the question of whether pair-

ing failure alone can account for the rare recombination observed between corresponding Zea and Tripsacum segments.

It is of considerable interest that many of the cells observed in which pairing failure occurred might have been interpreted as zygotene stages by one unfamiliar with the usual range of length of corn chromosomes at pachytene, and with the typical appearance of the corn nucleus at very early meiotic prophase. The fact that pairing failure appears to have increased rather than decreased with progressive shortening of the chromosomes further argues against a zygotene interpretation. The question now naturally arises whether most or all of the material previously described by others as zygotene may have been erroneously interpreted. Such stages of synapsis in progress as have been figured may actually represent synaptic failure or the opening of previously synapsed chromosomes. Material illustrative of "zygotene" is difficult to find, and searchers may have inadvertently selected organisms or cells more or less unbalanced or atypical in their pachytene pairing behavior for synapsis in progress. Synapsis may, in fact, usually be completed during the period traditionally considered premeiotic interphase. It is at interphase, after all, that chromosomes achieve their greatest linear extension. Such a finding would render considerably more plausible the idea that crossing over occurs at the time of chromosome duplication, since it now appears likely that duplication occurs during interphase (TAYLOR 1957).

SUMMARY

1. Cytogenetic studies were conducted of disomic plants heterozygous for a Zea-Tripsacum interchange chromosome 2 in which the segment derived from Tripsacum was equal in length to the portion of Zea chromosome 2S for which it had been exchanged, and carried loci normally present in this region. It was marked by a terminal knob.

2. Genetic recombination between the corresponding Zea and Tripsacum segments was found to be very rare.

3. These segments were commonly paired at pachytene, but a variable extent of pairing failure between them was found in about seven percent of microsporocytes.

4. Surprisingly large amounts of terminal pachytene pairing failure were found in each of the other chromosomes of the complement (average about three percent per chromosome end) although the extent and frequency of failure for the region known to be heterozygous for Zea and Tripsacum segments markedly exceeded all others. Ten apparently normal bivalents were present at diakinesis and metaphase I. A detailed analysis was made of the chromosome pairing failures found at pachytene.

5. The extent and frequency of pairing failures for all chromosomes varied greatly among anthers of a single flower and among flowers on the same tassel branch. Almost all anthers contained some cells in which all chromosomes were paired throughout their length, and all plants contained microsporocytes in which there was some pairing failure.

6. Positive correlations (significant at one percent level) were noted for relative chromosome length with frequency and extent of pairing failure. Negative correlations (significant at one percent level) were found for stage of advancement (as measured by total length of chromosomes) with extent and frequency of pairing failure suggesting that dissociation occurred during pachytene.

7. The frequencies of cells with various numbers of terminal pairing failures indicated a contagious pattern with higher than expected frequencies of cells with larger numbers of failures, assuming failures to be independent and equally probable.

8. It is suggested that much of the pairing failure observed here may be the result of irregular dissociation of homologous parts in a slightly unbalanced chromosome complement. Caution is advised in interpretation of isolated observations of pairing failure as indicating lack of homology of the parts involved.

9. It is also suggested that most or all of the material previously described as zygotene may represent erroneously interpreted synaptic failure or precocious opening, and that synapsis may usually occur during the period traditionally considered premeiotic interphase. It is pointed out that the idea that crossing over occurs during chromosome duplication would be rendered more plausible by such a finding.

LITERATURE CITED

BARBER, H. N., 1941 Chromosome behavior in Uvularia. J. Genet. **42**: 223–257.

BEADLE, G. W., 1933 Further studies of asynaptic maize. Cytologia **4**: 269–287.

EMERSON, R. A., G. W. BEADLE and A. C. FRASER, 1935 A summary of linkage studies in maize. Cornell Univ. Agr. Exptl. Sta. Mem. **180**: 1–83.

HUSKINS, LEONARD C., and E. MARIE HEARNE, 1933 Meiosis in asynaptic dwarf oats and wheat. J. Roy. Microscop. Soc. **53**: 109–117.

MAGUIRE, MARJORIE P., 1957 Cytogenetic studies of Zea hyperploid for a chromosome derived from Tripsacum. Genetics **42**: 473–486.

MANGELSDORF, P. C., and R. G. REEVES, 1939 The origin of Indian corn and its relatives. Tex. Agr. Exptl. Sta. Bull. **574**: 1–315.

MORGAN, D. T., 1956 Asynapsis and plasmodial microsporocytes in maize following X-irradiation of the pollen. J. Heredity **47**: 269–274.

REES, H., 1958 Differential behavior of chromosomes in Scilla. Chromosoma **9**: 185–192.

SADANAGA, K., 1957 Cytological studies of hybrids involving *Triticum durum* and *Secale cereale*. I. Alien addition races in tetraploid wheat. Cytologia **22**: 312–321.

TATE, MERLE W., and RICHARD C. CLELLAND, 1957 *Nonparametric and Short-cut Statistics*. Interstate Printers and Publishers, Danville, Illinois.

TAYLOR, J. H., 1957 The time and mode of duplication of chromosomes. Am. Naturalist **91**: 209–221.

STUDIES ON THE MUTANT MAROON-LIKE IN DROSOPHILA MELANOGASTER[1]

J. L. HUBBY[2] AND H. S. FORREST

Genetics Laboratory of the Department of Zoology, University of Texas, Austin, Texas

Received August 11, 1959

THE chemical constitution of a number of the pteridines that occur in Drosophila has now been determined, but there is still considerable ignorance about the structures of the red pigments of the eyes (the drosopterines), which, however, appear to be pteridines. The postulated precursors to these pigments have been structurally characterized (FORREST, HATFIELD and VAN BAALEN 1959). A discussion of the present knowledge of the red pigments has recently been given (VISCONTINI 1958).

The major metabolic pathways and the functions of the pteridines in this organism remain to be determined. The enzymatic conversion of 2-amino-4-hydroxypteridine to isoxanthopterin has been described (FORREST, GLASSMAN and MITCHELL 1956; NAWA, TAIRA and SAKAGUCHI 1958) and two mutants which lack this activity have been studied (FORREST, GLASSMAN and MITCHELL 1956; GLASSMAN and MITCHELL 1959). At present no other naturally occurring pteridine has been reported to undergo enzymatic conversion by extracts of this organism.

The two mutants, maroon-like and rosy^2, which lack the ability to convert 2-amino-4-hydroxypteridine to isoxanthopterin and hypoxanthine to xanthine to uric acid, are characterized by a dull brown eye color phenotype, reduced viability and abnormal accumulation and deficiencies of pteridines and purines (FORREST *et al.* 1956; HADORN and SCHWINCK 1956a,b; MITCHELL, GLASSMAN and HADORN 1959). The mutant rosy^2 has been shown to have a nonautonomous development of eye color (HADORN and SCHWINCK 1956a,b; HADORN and GRAF 1958). Thus rosy^2 eye anlagen implanted into wild type hosts develop a drosopterine phenotype identical to wild type. Moreover implants of wild type Malpighian tubes into rosy^2 hosts caused the drosopterine content in the host's head to approach that of wild type. Wild type eye discs implanted into rosy^2 hosts develop nonautonomously. maroon-like when tested as a heterozygote produced a gynandromorph exhibiting nonautonomous eye color development (GLASSMAN, unpublished). It should be added that maroon-like and rosy^2 are phenotypically indistinguishable and their pteridine and purine accumulations and deficiencies are identical.

The present paper is concerned primarily with the mutant maroon-like. In particular this sex-linked mutant has been more precisely located, quantitative

[1] Supported in part by the Robert A. Welch Foundation, Houston, Texas and in part by the National Institutes of Health Grant No. C-2269 to R. P. WAGNER.

[2] National Institutes of Health Predoctoral Fellow (1957–59).

measurements of the pteridines in this mutant and in wild type have been made, and the lack of xanthine dehydrogenase activity and the reduction in drosopterine content in the mutant have been studied. By the use of C^{14} labeled 2-amino-4-hydroxypteridine more precise information about the location of xanthine dehydrogenase and about the *in vivo* participation of the labeled compound has been obtained. The two mutants, which apparently control the synthesis of the same enzyme, afford a unique opportunity to study the level of enzyme activity in the heterozygous and double heterozygous condition.

MATERIAL AND METHODS

Linkage data

maroon-like (*ma-l*) was recovered in a single male offspring of an X-rayed wild type male (OLIVER, in BRIDGES and BREHME 1944). Preliminary mapping placed the mutant locus near vermilion (1–33.0). Further investigation indicated a locus to the right of Beadex (1–59.4) (GLASSMAN, unpublished). In a preliminary paper (GLASSMAN, HUBBY and MITCHELL 1958) it was reported that the progeny from females with a wild allele of maroon-like exhibit a maternal effect. The male progeny from a cross of an attached-X female (homozygous for the wild allele of maroon-like) with maroon-like males unexpectedly showed a wild type eye color although genetically they were maroon-like. Similarly all progeny of a cross of heterozygous maroon-like females with maroon-like males were phenotypically wild type even though a 1:1 ratio of wild type to maroon-like was expected.

The maternal effect limits the standard method of recombination analysis. This may be circumvented by an analysis of the chemotype (chemical expression of a genotype) obtained by paper chromatography. Thus the usual phenotypic classification of the markers employed in a cross can be supplemented by making a chromatogram of the classified individuals. The maroon-like genotype produces only trace amounts of isoxanthopterin while the wild type maroon-like allele produces easily discernible amounts of this compound. Thus a division of the classes is possible. Classification of this sort may be performed with any marker that contains the wild type amount of isoxanthopterin. Thus in the cross below, the eye color mutant raspberry^2 was phenotypically indistinguishable from the double mutant raspberry^2, maroon-like but chemotypically this double mutant lacked the wild type amount of isoxanthopterin that is characteristic of raspberry^2.

A preliminary cross was made as follows [where y = yellow (1–0.0), ct^6 = cut^6 (1–20.0), ras^2 = raspberry^2 (1–32.8), f^5 = forked^5 (1–56.7) and m = miniature (1–36.1)]:

$$P_1 \quad y\ ct^6\ ras^2\ f^5\ ♀♀ \times m\ ma\text{-}l\ ♂♂$$
$$F_1 \quad \frac{y\ ct^6\ ras^2\ f^5\ ♀♀ \times m\ ma\text{-}l\ ♂♂}{m\ ma\text{-}l}$$

The F_2 males were scored according to the procedure described above. The results

indicated that maroon-like was situated 12.7 ± 2 crossover units to the right of f^5 (1–56.7).

A more exhaustive analysis was then made using Beadex^3 (Bx^3) as the most distal, well located marker. There were 95 recombinations between maroon-like and Beadex^3 out of 1,212 individuals scored. This places the locus of maroon-like at 67.2 ± 0.7 on the X chromosome. This analysis also demonstrated that the mutant is about 0.7 as viable as Beadex^3 (fully viable).

The chemotype of maroon-like

The drosopterines: These pigments are a complex of at least three compounds (VISCONTINI, HADORN and KARRER 1957). Their pteridine nature was clearly demonstrated by FORREST and MITCHELL (1955), and has been recently confirmed (VISCONTINI *et al.* 1957). VISCONTINI (1958) designates the three components as drosopterine, isodrosopterine and neodrosopterine. A fourth pigment, red rather than orange, has been demonstrated in this work by electrophoresis. The chemical constitution of these compounds is unknown.

The mutant maroon-like clearly has reduced amounts of visible pigments in the eye. In order to determine how the three drosopterines and the red component are affected, the following experiment was performed. One to six heads of three-day-old Canton Special wild type males were squashed compactly on paper electrophoresis strips. The strips were placed in a Spinco Durrum Cell and a potential of 400 volts was applied to them for four hours. Two percent acetic acid was used as the electrolyte. The four components were well separated under these conditions. Standard curves for each pigment were made using a paper densitometer (Photovolt Model 525). Eight sets of ten, three-day-old maroon-like male heads were treated in the same manner, and the densitometer readings of the four components were recorded. These values were compared with the standard curves made from wild type heads and are represented in Figure 1 as the percentage of the pigments in the mutant as compared to wild type. Maternally affected maroon-like individuals were also tested in this manner (first and fourteenth day emergence). There is a simultaneous and proportionally equal reduction in all four of the pigments in the mutant, and maternally affected individuals have a complete restoration of these pigments on early emergence.

Other pteridines: Quantitative measurements were made of isoxanthopterin, 2-amino-4-hydroxypteridine and biopterin in wild type, maroon-like and maternally affected maroon-like (first and fourteenth day emergence). In these tests two dimensional chromatograms were necessary to separate 2-amino-4-hydroxypteridine from biopterin. Individual flies of an appropriate age were placed in boiling water for two minutes, dried on filter paper and squashed three millimeters from each edge on a 9 × 11″ sheet of Whatman's No. 1 chromatographic paper. Ascending chromatograms were developed in the first dimension with n-propanol:1% ammonia (2:1). The chromatograms were developed for 12 to 16 hours at room temperature. All chromatography and drying were done in the dark. After being run in the first dimension the chromatograms were air

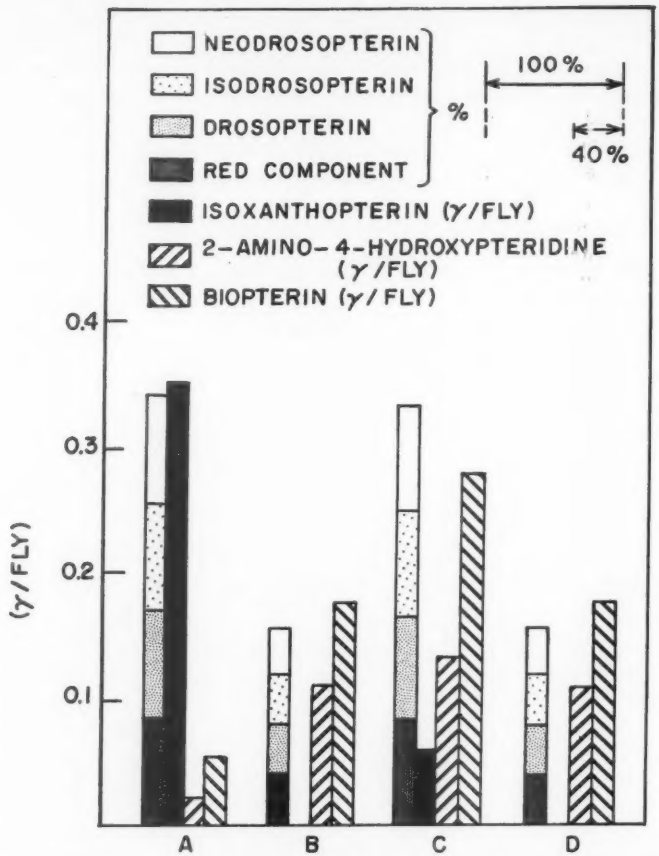

FIGURE 1.—Quantitative estimations of the pteridines in wild type and maroon-like male adults. A = One day old Canton Special wild type male. B = One day old maroon-like male. C = One day old maroon-like male from a wild type attached-X female. D = One day old maroon-like male from a wild type attached-X female from a culture fourteen days after first emergence. Isoxanthopterin was determined with a Corning No. 5860 filter (primary) and a Farrand 415 mμ interference filter plus a Corning No. 3389 (secondary). Biopterin and 2-amino-4-hydroxypteridine were determined with a Corning No. 5860 filter (primary) and a Farrand 454 mμ interference filter plus a Corning No. 3389 (secondary).

dried. The solvent used in the second dimension was five percent acetic acid. This solvent, while tending to spread the fluorescent materials has the advantage of separating 2-amino-4-hydroxypteridine from biopterin.

Quantitative measurements were made as follows: the fluorescent materials were viewed with a Mineral-Light ultraviolet source (principle emission 365

mμ), the appropriate areas were circled, marked, and the paper was cut out and placed in 10 mm test tubes. Two milliliters of one percent ammonia were added to each tube and the material was allowed to stand for three hours (with occasional shaking). Blanks cut from the same paper as the sample were employed at all times. Standard curves for each of the three compounds were made by placing known amounts of the pure compounds on chromatographic paper and performing the same chromatographic techniques that had been used for the biological material.

All fluorescent measurements were performed with a Farrand Model A Fluorometer. The data is given in Figure 1. It can be seen that concomitant with the drop in red pigments and the total elimination of isoxanthopterin, there is a marked increase in 2-amino-4-hydroxypteridine and biopterin. In the maternally affected individuals (first day emergence) the red pigments are present in normal amounts; there is a trace amount of isoxanthopterin, and 2-amino-4-hydroxypteridine and biopterin are still relatively elevated. There is no maternal effect in maroon-like individual from 14-day-old cultures.

A fifth pteridine of interest in this study, sepiapteridine, was impossible to measure accurately because of its photolability, adhesion to chromatographic paper and lack of a pure standard. From visual inspection of chromatograms, maroon-like showed a three- to four-fold accumulation of this compound when compared to wild type.

Injection of isoxanthopterin, xanthine and uric acid into maroon-like flies

In maternally affected males from heterozygous maroon-like females or homozygous wild type attached-X females, the complete restoration of the drosopterine content is observed while traces of isoxanthopterin and uric acid are present. Since these are products of the action of xanthine dehydrogenase in this organism and since it has been shown that traces of xanthine dehydrogenase are present in maternally affected maroon-like individuals (GLASSMAN and MITCHELL 1959b), it was thought that the restoration of red pigment might be due to the presence of these compounds. The minute quantities present in the maternally affected flies suggest that any possible contribution would be of a catalytic nature. Such a suggestion for isoxanthopterin and uric acid has been made (FORREST and MITCHELL 1955). Accordingly maroon-like male third instar larvae were injected with isoxanthopterin, xanthine and uric acid.

Saturated solutions of the three compounds were injected into the larvae employing modifications of the older method (EPHRUSSI and BEADLE 1936) suggested by H. K. MITCHELL (to be published) and one of the authors. After injection the larvae were washed thoroughly and placed on moist filter paper in a food vial where they were allowed to develop. Control larvae were squashed directly after washing, on chromatography paper to determine the amounts of the compounds actually injected. After emergence the phenotype was determined and then the fly was chromatographed in the usual manner.

Isoxanthopterin was located on the chromatogram in the usual manner and the quantity of this material present was determined fluorometrically. Uric acid

was detected by spraying the chromatograms with N,2,6-trichloro-quinoneimine (BRAY, THORPE and WHITE 1950). Xanthine was not determined in injected flies because of the lack of a sensitive specific test. In the compounds tested for, there was no difference in the amount of the material present in emerged adults and the amount present in the larval controls. The quantities observed in the adult flies were comparable or exceeded the amount present in maternally affected individuals. There was no detectable change in drosopterine content in any of the experimental material.

Injection of C^{14} labeled 2-amino-4-hydroxypteridine into wild type larvae

The metabolic relationship of the simpler pteridines i.e., 2-amino-4-hydroxypteridine, xanthopterin and isoxanthopterin to the pteridines with side chains at the 6 position is unknown. In an effort to determine whether 2-amino-4-hydroxpteridine served a role other than that of presumed precursor to isoxanthopterin (FORREST, GLASSMAN and MITCHELL 1956), this compound was synthesized through 2-amino-4-hydroxy-6-tetrahydroxybutylpteridine from D-glucose-U-C^{14} (FORREST and WALKER 1949). The tetrahydroxy compound was oxidized to the 6-carboxy compound which was in turn converted to 2-amino-4-hydroxypteridine by the action of sunlight. This results in uniform labeling of the 6 and 7 positions.

A saturated solution of this material was injected into 25 wild type third instar larvae. Nineteen adults emerged and were squashed in one compact spot on Whatman's No. 3 chromatographic paper. A descending one dimensional chromatogram was made using n-propanol: 7% ammonia (2:1) as solvent. Using the radioautographic technique and allowing contact between the X-ray plate and the chromatogram for 21 days only two areas of radiation were observed. These corresponded to the fluorescent areas of 2-amino-4-hydroxypteridine and isoxanthopterin. As a further check the areas corresponding to the drosopterines, isoxanthopterin, sepiapteridine, 2-amino-4-hydroxypteridine, biopterin and all the nonfluorescing areas were cut out and eluted into planchets. The amount of radioactive material present in these samples was determined on a Nuclear Chicago Counter. Table 1 clearly shows that isoxanthopterin only arises from 2-amino-4-hydroxypteridine. No radioactivity is incorporated into any of the other pteridines.

A second experiment using two dimensional chromatography (same solvents as before) and radioautography for periods up to seven weeks produced the same results. The first condensation product in the chemical synthesis of 2-amino-4-hydroxypteridine (2-amino-4-hydroxy-6-tetrahydroxybutylpteridine) was also injected into larvae. As revealed by the same techniques, no metabolic transformation of the compound occurred.

Location of xanthine dehydrogenase in Drosophila

The report of HADORN and SCHWINCK (1956a) on the mutant rosy^2 indicates clearly nonautonomous development in drosopterine biosynthesis. The authors

TABLE 1

Distribution of carbon^{14} labeled 2-amino-4-hydroxypteridine following injection into wild type larvae

Pteridine	Counts per minute*
Drosopterines	0
Isoxanthopterin	137
Sepiapteridine	0
2-amino-4-hydroxypteridine	168
Biopterin	0
All other areas	0

* Corrected for background.

concluded that an agent, "rosy^2 Stoffe", found outside the eye plays an important role in the formation of the drosopterines and that the mutant rosy^2 is unable to produce this agent in normal amounts. Apparently wild type Malpighian tubes can produce sufficient quantities of "rosy^2 Stoffe" themselves to affect the synthesis of the drosopterines in a rosy^2 host. The phenotypic and chemotypic identity as well as the lack of the same enzyme in maroon-like and rosy^2 (also the gynandromorph nonautonomy of maroon-like) have already been stated. Thus it seems reasonable to conclude that the drosopterine reduction in maroon-like is also the result of a deficiency in a material similar or identical to "rosy^2 Stoffe". A reasonable deduction would be that the enzyme and "rosy^2 Stoffe" are identical.

Hence it was of interest to know if there is a localization of xanthine dehydrogenase in an organ or organ system, particularly in the Malpighian tubes. For added accuracy and because of the small size of the organs, 2-amino-4-hydroxypteridine-6, 7-C^{14} was used as substrate for the assay of the enzyme in these experiments. One-day-old wild type males were etherized and chilled in a beaker of ice. The flies were dissected singly in a drop of 0.1 M "Tris" buffer pH 8.0. The various organs were removed to individual chilled Ten Broeck grinders. Any mutilated organ was rejected. After each dissection the remainder of the fly and the buffer used for dissection were also placed in a chilled grinder. Twenty individuals were dissected for each experiment. Next the different parts were thoroughly ground in 0.5 ml 0.1 M "Tris" buffer at pH 8.0. Twenty undissected individuals ground in the same manner were used as a control for total enzyme activity. Boiled ground flies were used in each case for blank measurements; 1 μg of 2-amino-4-hydroxypteridine-6, 7–C^{14} and 2 μg diphosphopyridine nucleotide (abbreviated DPN$^+$ hereafter) were added to all samples. The mixture was incubated for one hour at 25°C. Then 1 μg of unlabeled isoxanthopterin was added to each sample. The samples were placed on Whatman's No. 3 chromatographic paper and developed in a descending chromatogram with n-propanol:7% ammonia (2:1). After location of the substrate and product by their fluorescence, they were eluted from the paper and their radioactivities determined as before.

In the first experiment the heads, testes (with accessory gland), gut and the remainder of the fly including the Malpighian tubes were tested separately. Essentially all the activity was found in the remainder (Table 2). In the next ex-

TABLE 2

Xanthine dehydrogenase activity of various organs in wild type Drosophila as measured by the production of isoxanthopterin from carbon-14 labeled 2-amino-4-hydroxypteridine

Organ	Pteridine	Counts per minute*
	Experiment 1	
Head	2-amino-4-hydroxypteridine	741
	isoxanthopterin	9†
Testes	2-amino-4-hydroxypteridine	694
	isoxanthopterin	0
Gut	2-amino-4-hydroxypteridine	711
	isoxanthopterin	13†
Rest	2-amino-4-hydroxypteridine	455
	isoxanthopterin	76±7
Whole	2-amino-4-hydroxypteridine	562
	isoxanthopterin	99±9
	Experiment 2	
Malpighian tubes	2-amino-4-hydroxypteridine	643
	isoxanthopterin	17†
Rest	2-amino-4-hydroxypteridine	583
	isoxanthopterin	92±8
Whole	2-amino-4-hydroxypteridine	512
	isoxanthopterin	108±8

* Corrected for background.
† Not significant.

periment only the Malpighian tubes were removed from the flies. Again the two parts of the animal were assayed separately. Little activity is accounted for by the Malpighian tubes (Table 2).

Xanthine dehydrogenase

Forrest, Glassman and Mitchell (1956) demonstrated that extracts of wild type stocks of *Drosophila melanogaster* contain enzyme activity for the conversion of 2-amino-4-hydroxypteridine to isoxanthopterin, xanthine to uric acid and xanthopterin to leucopterin. Later, Glassman and Mitchell (1959) showed that this activity can be ascribed to a xanthine dehydrogenase rather than to a xanthine oxidase because well dialyzed and purified preparations require methylene blue or DPN^+ for activity. Nawa, Taira and Sakaguchi (1958) drew the same conclusion. In this report it was found that extracts treated with sufficient activated charcoal to remove the last traces of fluorescent materials were essentially devoid of activity without adding an electron acceptor such as DPN^+.

Assay method

In a preliminary study for an assay system, it was decided to measure the increase in optical density at 340 mμ resulting from the reduction of DPN^+ to

reduced diphosphopyridine nucleotide (abbreviated DPNH hereafter) during the reaction. Zero to one-day-old adult flies were used at all times. The flies were stored at −20°C for periods up to several weeks. No loss of activity was noted with flies stored three or four months; however, flies stored for a period of one year had lost 75 per cent of their activity.

For enzyme extraction one part of the frozen flies was ground with five parts (w/v) ice cold 0.1 M "Tris" buffer, at pH 8.0, in a Ten Broeck grinder. The flies were ground for three or four minutes with precaution taken to keep the grinder and its contents ice cold. The slurry was then centrifuged in a Spinco refrigerated centrifuge for 20 minutes at 20,000 rpm. Next the supernatant was carefully removed from between the lipid layer and the pellet, and treated with charcoal.

Charcoal treatment

Drosophila contains relatively large amounts of different pteridines and purines which must be removed before assaying extracts for xanthine dehydrogenase activity. Dialysis against repeated changes of buffer necessitates up to 48 hours of treatment before the last traces of fluorescent materials are removed. This prolonged dialysis results in a 20 to 50 percent yield of enzyme activity as compared to charcoal treated controls. This procedure was discarded as a routine method. It was found that the addition of Darco Grade 60 activated charcoal at 0.5:1 (w/w) to flies effectively removed all coloring matter and fluorescent material from the extract. Little or no loss of activity results from this treatment as judged by production of isoxanthopterin from excess 2-amino-4-hydroxypteridine in treated and untreated material.

In practice the supernatant from the first centrifugation was slowly poured into a centrifuge tube containing the activated charcoal. This suspension was slowly mixed and allowed to stand for several minutes at 0°C. Then this mixture was centrifuged for 20 minutes at 20,000 rpm. The final supernatant was removed and used as the source of the enzyme.

0.1 M "Tris" buffer was used at all times. The assay mixture contained from 0.1 to 0.2 ml extract (1 to 2 mg protein/ml) in a total volume of one milliliter. Under the conditions of the experiments there is no measurable reduction of DPN^+ without addition of substrate (hypoxanthine or xanthine). The charcoal treated extracts do not oxidize added DPNH at any appreciable rate unlike untreated extracts (Negelein and Schon 1957). Activity is expressed by positive change in optical density at 340 mμ/minute/mg protein. The protein was routinely analyzed by the method of Lowry, Rosebrough, Farr and Randall (1951).

All final determinations were made using a Beckman DU Spectrophotometer with an ERA recording attachment. Hypoxanthine was used as substrate. Xanthine is also oxidized under these conditions but at approximately one third the rate of hypoxanthine. These rates were determined during the first three or four minutes after addition of the enzyme while the process was linear. The temperature was controlled at 35°C. The pH optimum for the reaction is near

8.0. The optimum concentration for DPN^+ is unity with respect to hypoxanthine concentration. The reaction rate increases with increase of substrate concentration to 1.5×10^{-3} M hypoxanthine. Within limits the reaction is proportional to enzyme concentration.

For the analysis of the level of enzyme activity in different genotypes, the following large scale crosses were made: Canton Special wild type females with maroon-like males; wild type females with $rosy^2$ males; maroon-like females with $rosy^2$ males. The cultures were raised at 25°C. The female progeny from the above crosses were collected for three days and stored in a deep freeze for further use. They have been used exclusively in this analysis. The extracts were prepared as stated above. Several substrate concentrations were used, and they were all mutually consistent. The data reported here is for the optimum concentration of 1×10^{-3} M. In every assay three identical estimations were made for each genotype. The assay was repeated a number of times with reasonable consistency between them. Results are given in Table 3.

TABLE 3

Xanthine dehydrogenase level in different genotypes

Genotype	Activity*	Ratio to wild type
+/+; +/+	0.110	1.0
ma-l/+; +/+	0.073	.67
+/+; ry^2/+	0.058	.53
ma-l/+; ry^2/+	0.040	.36
+/+; *In*†/+	0.049	.44
+/+; *In*/ry^2	0.010	.09

* Positive change in optical density at 340 mμ/minute/ milligram protein and in agreement with several independent determinations.
† *In (3RC; 3LP) Sb* e^s.

It was noted in a cross between $rosy^2$ females with *In (3RC;3LP) Sb* e^s males that all progeny carrying the Stubble (*Sb*) marker were phenotypically $rosy^2$. Subsequent crosses involving this inversion repeatedly gave the same results. Quantitative estimation of the pteridines in the heterozygotes revealed no difference in chemotype between them and $rosy^2$ homozygotes. The inversion heterozygous with wild type reveals no substantial difference between this inversion and wild type.

Salivary gland chromosome analysis of the inversion shows breaks in the left arm in the middle of 63 C and at 72 E 1–2 and in the right arm just before 92 E and following the medium doublet 100 F 1–2 (MORGAN, BRIDGES and SCHULTZ 1937). The locus for $rosy^2$ has not been determined with exactitude. HADORN and GRAF (1958) state its location as 3–51 ± 8. Consequently the possibility exists that one of the breaks (more likely the break at 92 E) in the inversion could be at or very near the $rosy^2$ locus thus resulting in a deletion or alteration of the gene. Another possible explanation of this interaction is that the inversion could be carrying a mutant allele of $rosy^2$. Unfortunately the inversion carries a recessive

lethal factor and this possibility cannot be tested as yet. However, individuals heterozygous for the inversion and for rosy^2 or wild type could be tested for xanthine dehydrogenase activity. The flies were collected as before and assayed for enzyme activity. Results are presented in Table 3.

DISCUSSION

In part the chemotype in the mutant maroon-like can be explained by the lack of xanthine dehydrogenase activity in the mutant. The increase of 2-amino-4-hydroxypteridine and hypoxanthine and the absence of isoxanthopterin and uric acid are a direct result of the lack of enzyme. However the increase in biopterin and in sepiapteridine are not directly explainable by the lack of the enzyme. The reduction, but not lack of drosopterines in maroon-like concomitant with the lack of isoxanthopterin and uric acid make it unlikely that either of these compounds is a precursor to the drosopterines. The lack of response to injected isoxanthopterin, xanthine and uric acid lends further support to this assertion and negates the idea that they are involved catalytically in the formation of the red pigments. HADORN and GRAF (1958) report that injection of isoxanthopterin into the rosy^2 mutant has no effect on the drosopterine content. The nonlabeling of the drosopterines or any other complex pteridine component in Drosophila following injection of C^{14} labeled 2-amino-4-hydroxypteridine rules out this compound as a precursor to the red pigments or any other pteridine except isoxanthopterin.

The simultaneous and proportionally equal reduction of the three drosopterines and the red component suggests either a common precursor for all four of these components or a common reaction leading to their formation. Evidence for the first suggestion has been obtained (VISCONTINI 1958). The three drosopterines are interconvertible when in a reduced form, thus suggesting the possibility of a common intermediate to these pigments. Support for the second suggestion was gained by the study of the accumulation of the two yellow pigments in the mutant sepia (FORREST, HATFIELD and VAN BAALEN 1959). The major component, sepiapteridine, and the second pigment are present in great excess over the amounts found in wild type flies. There is a complete absence of red pigments in this mutant (HADORN and MITCHELL 1951). But until the structure of the drosopterines are known, these relationships are still obscure.

If an intermediate or a component of a common reaction step in the biosynthesis of the drosopterines is available in reduced amounts in maroon-like and rosy^2, its identity and causal relationship to the lack of xanthine dehydrogenase is unknown. The equation of "rosy^2 Stoffe" with the enzyme seems to be unwarranted on the basis of the low enzyme level in the Malpighian tubes.

One common feature between the lack of xanthine dehydrogenase activity and the reduction of drosopterine content in maroon-like and rosy^2 should be pointed out. In the reactions, 2-amino-4-hydroxypteridine → isoxanthopterin and hypoxanthine →xanthine → uric acid, DPN^+ has been shown to be an obligate cofactor in Drosophila (GLASSMAN, FORREST and MITCHELL 1957; NAWA, TAIRA and SAKAGUCHI 1958; and this study). This conclusion was drawn from both

in vitro and *in vivo* studies. DPNH is produced by these reaction steps. If one of the reactions in the biosynthesis of the drosopterines required DPNH for its occurrence, the quantity of this material *at the site of pigment synthesis* might be limited in both maroon-like and rosy^2. There are several features of this suggestion that make it reasonable. First, it has been demonstrated that the *in vitro* interconversion reactions of the drosopterines only occur when these compounds are in the reduced form (VISCONTINI 1958). Many reactions of the 6-substituted pteridines in algae probably take place when these compounds are in a reduced form (FORREST, unpublished). Secondly, the major formation of isoxanthopterin occurs during the first 60 hours of pupal existence i.e., immediately before drosopterine biosynthesis (HADORN and MITCHELL 1951). Also, although there would be a reduction in the amount of DPNH at that time in the mutants, there would certainly not be a complete lack of the compound. Thus this would reasonably explain the reduction but not the lack of the drosopterines in the mutants.

The limiting reduction potential resulting from the absence of xanthine dehydrogenase could cause an accumulation of the substrates for the final stages in the conversion of sepiapteridine (primarily) and perhaps biopterin (secondarily) which might therefore accumulate. Since xanthine dehydrogenase is apparently ubiquitous in wild type, the immediate implication of this scheme should be the requisite of substrate (2-amino-4-hydroxypteridine or hypoxanthine) location at the pigment forming site to couple with the synthesis of the drosopterines. The introduction of the Malpighian tubes into rosy^2 flies greatly increases the isoxanthopterin concentration and restores the wild type drosopterine content (HADORN and GRAF 1958). The production of DPNH from isoxanthopterin synthesis would allow full drosopterine formation. This would imply the identity of "rosy^2 Stoffe" and 2-amino-4-hydroxypteridine. In the maternally affected individuals in which it has been shown that some enzyme is present (GLASSMAN and MITCHELL 1959b) it can be visualized that the reducing power controlled by the substrate is just sufficient for the full synthesis of the drosopterines.

With regard to the enzyme activity in the heterozygotes, little work has been done in studies of this sort (SAWIN and GLICK 1943; RUSSELL and RUSSELL 1948; FOSTER 1951). Apparently in the rosy^2 heterozygote, the two alleles on homologous chromosomes act independently of each other in controlling enzyme activity. In the maroon-like heterozygote consistently higher than one half the activity of wild type resulted. It is interesting that this occurs with a sex-linked mutant locus where the locus is normally present as a hemizygote in the male. In the double heterozygous condition there is further reduction of activity. This indicates the possibility of a sequential relationship in the formation of the enzyme by the two different loci. On the basis of the persistence of the maternal effect in progeny from females homozygous for rosy^2 and heterozygous for maroon-like, GLASSMAN has proposed the following:

$$X \xrightarrow{\text{rosy}^2} Y \xrightarrow{\text{maroon-like}} \text{Enzyme}$$

The findings in this work do not conflict with this arrangement.

The very interesting interaction of the *In (3RC;3LP) Sb* e^s inversion with the rosy^2 locus will make profitable further work on this relation. It may be possible to "break up" this inversion by crossing over and determine what area of the chromosome is involved in disturbing the xanthine dehydrogenase activity.

SUMMARY

The chemotype (chemical expression of a genotype) of the mutant maroon-like in *Drosophila melanogaster* has been analyzed and compared to wild type. The influence of the maternal parent carrying a wild allele of maroon-like on its maroon-like progeny has been studied and the mutant has been relocated at the extreme right end of the X chromosome in *Drosophila melanogaster*. The relation between the lack of xanthine dehydrogenase and the reduction of red eye pigment in the mutant is discussed. The lack of response to injected products of xanthine dehydrogenase is reported, and the hypothesis is advanced for the identity of "rosy^2 Stoffe" and DPNH. C^{14} labeled 2-amino-4-hydroxypteridine is converted *in vivo* into isoxanthopterin in the organism but not into any other compound.

In analyzing the level of xanthine dehydrogenase activity in the following heterozygotes: maroon-like; rosy^2; and maroon-like and rosy^2, it was found that rosy^{2+} acted independently of its homologous allele, while maroon-like exceeded the half maximal level. The double heterozygote had a multitive effect arguing for a sequential control of the two genes for the enzyme activity.

The inversion *In(3RC;3LP) Sb* e^s is shown to have an effect on the activity of xanthine dehydrogenase.

ACKNOWLEDGMENT

The authors wish to express their gratitude to DR. ROBERT P. WAGNER for his advice and constructive criticisms throughout the course of this work and for the help in the preparation of the manuscript.

LITERATURE CITED

BRAY, H. G., W. V. THORPE, and K. WHITE, 1950 The fate of certain organic acids and amides in the rabbit. Part 10. The application of paper chromatography to metabolic studies of hydroxybenzoic acids and amides. Biochem. J. **46**: 271–275.

BRIDGES, C. B., and K. S. BREHME, 1944 *The mutants of Drosophila melanogaster*. Carnegie Inst. Wash. Publ. **552**: 257 pages.

EPHRUSSI, B., and G. W. BEADLE, 1936 A technique of transplantation for Drosophila. Am. Naturalist **70**: 218–225.

FORREST, H. S., E. GLASSMAN, and H. K. MITCHELL, 1956 Conversion of 2-amino-4-hydroxypteridine to isoxanthopterin in *Drosophila melanogaster*. Science **124**: 725–726.

FORREST, H. S., D. HATFIELD, and C. VAN BAALEN, 1959 Characterization of a second yellow compound from *Drosophila melanogaster*. Nature **183**: 1269–1270.

FORREST, H. S., and H. K. MITCHELL, 1955 Pteridines from Drosophila. III. Isolation and identification of three more pteridines. J. Am. Chem. Soc. **77**: 4856–4869.

FORREST, H. S., and J. WALKER, 1949 The condensation of 2:4:5-triamino-6-hydroxypyrimidine with glucose and fructose. J. Chem. Soc. **16**: 79–85.

FOSTER, M., 1951 Enzymatic studies of pigment-forming abilities in mouse skin. J. Exptl. Zool. **117**: 211–246.

GLASSMAN, E., H. S. FORREST, and H. K. MITCHELL, 1957 Genetic control of xanthine dehydrogenase in *Drosophila melanogaster*. Genetics **41**: 566 (Abstr.).

GLASSMAN, E., J. L. HUBBY, and H. K. MITCHELL, 1958 Maternal effect of *ma-l*$^+$ in *Drosophila melanogaster*. Proc. 10th Intern. Congr. Genet. **2**: 98 (Abstr.).

GLASSMAN, E., and H. K. MITCHELL, 1959a Mutants in *Drosophila melanogaster* deficient in xanthine dehydrogenase. Genetics. **44**: 153–162.

1959b Maternal effect of *ma-l*$^+$ on xanthine dehydrogenase of *Drosophila melanogaster*. Genetics **44**: 547–554.

HADORN, E., and G. E. GRAF, 1958 Weitere Untersuchungen uber den nichtautonomen Pterinstoff-wechsel der Mutante rosy von *Drosophila melanogaster*. Zool Anzeiger **160**: 231–243.

HADORN, E., and H. K. MITCHELL, 1951 Properties of mutants of *Drosophila melanogaster* and changes during development as revealed by paper chromatography. Proc. Natl. Acad. Sci. U. S. **37**: 650–665.

HADORN, E., and I. SCHWINCK, 1956a A mutant of Drosophila without isoxanthopterin which is nonautonomous for the red eye pigments. Nature **177**: 940–941.

1956b Fehlen von Isoxanthopterin und Nicht-Autonomie in der Bildung der roten Augenpigmente bei einer Mutante (rosy^2) von *Drosophila melanogaster*. Z. Ind. Abst. Vererb. **87**: 528–553.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL, 1951 Protein measurement with the Folin Phenol Reagent. J. Biol. Chem. **193**: 265–275.

MITCHELL, H. K., E. GLASSMAN, and E. HADORN, 1959 Hypoxanthine in rosy and maroon-like mutants of *Drosophila melanogaster*. Science **129**: 268–269.

MORGAN, T. H., C. B. BRIDGES, and J. SCHULTZ, 1937 Constitution of the germinal material in relation to heredity. Carnegie Inst. Wash. Ybk. **36**: 301.

NAWA, S., T. TAIRA, and B. SAKAGUCHI, 1958 Pterine dehydrogenase found in *Drosophila melanogaster*. Proc. Japan Acad. **34**: 115–119.

NEGELEIN, E., and R. SCHON, 1957 Reduced diphosphopyridine nucleotide dehydrogenase enzyme system in *Drosophila melanogaster*. Biokhimiya **22**: 191–201.

RUSSELL, L. B., and W. L. RUSSELL, 1948 A study of the physiological genetics of coat color in the mouse by means of the dopa reaction in frozen sections of skin. Genetics **33**: 237–262.

SAWIN, R. B., and D. GLICK, 1943 Atropinesterase, a genetically determined enzyme in the rabbit. Proc. Natl. Acad. Sci. U. S. **29**: 55–59.

VISCONTINI, M., 1958 Fluoreszierende Stoffe aus *Drosophila melanogaster*. 10. Beitrag zur Konstitutions aufklärung der Drosopterine. Helv. Chim. Acta **41**: 1299–1304.

VISCONTINI, M., E. HADORN, and P. KARRER, 1957 Fluoreszierende Stoffe aus *Drosophila melanogaster*. 5. Der roten Augenfarbstoffe. Helv. Chim. Acta **40**: 579–585.

HETEROSIS IN CROSSES OF INBRED LINES OF RATS[1]

J. F. KIDWELL[2], H. J. WEETH[2], W. R. HARVEY[3], L. H. HAVERLAND[2], C. E. SHELBY[4], AND R. T. CLARK[4]

University of Nevada, Reno, Nevada, and U. S. Dept. of Agriculture

Received August 17, 1959

HETEROSIS has been the subject of intense theoretical and applied research for more than 50 years, largely because of its important implication for both evolution and breeding practice. Among the mammals heterosis has been observed as a result of crossing breeds, strains or inbred lines of cattle, sheep, swine, rabbits, guinea pigs, rats, and mice. A detailed review of the vast literature compilation concerning heterosis in mammals is neither possible nor desirable in this paper. Reference is made only to those papers relevant to this experiment.

The theoretical aspects of the problem of specific and general combining ability in swine have been investigated by HENDERSON (1948, 1949, 1952). DICKERSON (1952) considered the use of inbred lines for heterosis tests with particular reference to swine, and presented an interpretation of the evidence concerning the sort of genetic system underlying economically important traits. He also compared the effectiveness of several alternative breeding methods.

The experiment described herein was devised to study the degree of heterosis, general and specific combining ability, sex linkage, and maternal influence arising from crosses among four inbred lines of rats. The design and analysis was largely predicated on the cited work of HENDERSON and DICKERSON. The data refer to 28-day (weaning) and 70-day body weight.

MATERIALS AND METHODS

Four inbred lines were available for this investigation. An albino line, designated B, was obtained from the Department of Genetics, University of California, Berkeley. An albino and a black line were obtained from the Department of Animal Husbandry, University of California, Davis, and are referred to as A and D, respectively. The fourth line, designated H, was black hooded, and was developed in this laboratory from a commercial stock. Inbreeding coefficients were not exactly determined, but were estimated to average above 60 percent in

[1] This investigation was supported in part by Cancer Research Funds of the University of California, and was conducted in cooperation with the Animal Husbandry Research Division, Agricultural Research Service, U. S. Department of Agriculture, under Western Regional Project W-1, "The Improvement of Beef Cattle Through the Application of Breeding Methods."

[2] Department of Animal Husbandry, University of Nevada, Reno. This work was completed while the senior author was on leave with the Department of Genetics, University of California, Berkeley.

[3] Biometrician, In Charge, Livestock Research Staff, Biometrical Services, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.

[4] U. S. Department of Agriculture, Denver, Colorado.

all lines and approached 90 percent in some, the Davis rats being the most highly inbred and the hooded rats the least.

A standard complete ration developed in this laboratory was fed to all animals. Females were first mated after 100 days of age. First litters only were used in this study. Litters were reduced to six at five days. An attempt was made to retain three males and three females, but no selection was practiced within sexes. Litters were weaned and weighed at 28 days. The rats were fed in like-sexed groups of three or six, depending on cage size, from 28 through 70 days when a final weight was taken. A single unshrunk weight was used. No artificial selection with respect to body size had been applied to the lines used in this study.

All possible crosses, including linebreds and reciprocals, were made among the four lines. At least four litters were produced in each of the 16 categories with a total of 104 litters. Litter averages for each sex rather than individual weights were used in the analysis. Hence, there were 208 observations each for 28- and 70-day weight.

The mathematical model used in the analysis was an extension of the one formulated by HENDERSON (1948) to include the effects of sex, heterosis, and linebreds as follows:

$$y_{ijklm} = \mu + s_i + h_j + p_{1k} + g_{2k} + g_{2l} + d_{2kl} + r_{2kl} + m_{2l} + e_{ijklm}$$

where:

y_{ijklm} = the average weight (28- or 70-day) for the mth litter in the cross (or linebred) obtained by crossing the kth line of sire with the lth line of dam and for the ith sex. The j subscript identifies whether the result obtained is an inbred line ($j = 1$) or a cross ($j = 2$)

μ = the general mean when equal numbers exist with respect to all effects in the model

s_i = the effect of the ith sex

h_j = the effect of the jth type of cross, i.e., linebred or cross

p_{1k} = the kth linebred within the linebreds

g_{2k} = the general combining ability effect from the kth line of sire

g_{2l} = the general combining ability effect from the lth line of dam

d_{2kl} = the specific combining ability for the kl or lk cross

r_{2kl} = the sex-linked effect for the kth line of sire mated to the lth line of dam

m_{2l} = the maternal effect for the lth line of dam measured over the crosses only. In fact, the g's, d's, r's, and m's are measured from the crosses only

e_{ijklm} = an effect common to all sex-litter subclasses

All constants were obtained by a conventional least squares analysis and all tests of significance were made under the assumption that all effects (except the set being tested) did exist.

RESULTS AND DISCUSSION

The analysis of variance of 28- and 70-day weights is presented in Table 1. The least squares means for the various comparisons are presented in Table 2. Since specific comparisons would be of extremely limited interest, a test of significance

TABLE 1

Analysis of variance in 28- and 70-day weights (mean squares only)[1]

Source of variation	d.f.	Mean squares: 28-day weight	Mean squares: 70-day weight
Sexes (S)	1	543.4**	204,992**
Heterosis (H)	1	211.6	2,777*
Linebreds (L)	3	628.9**	9,146**
General combining abilities (G)	3	353.4**	1,114
Specific combining abilities (D)	2	90.2	241
Sex linkage effects (R)	3	89.4	907
Maternal effects (M)	3	577.7**	4,425**
Error	191	70.5	655

[1] Adjusted for unequal frequencies by least squares.
* Significant at .05 level of probability.
** Significant at .01 level of probability.

TABLE 2

Least squares means

Classification	No.	28-day weight	70-day weight
Sexes ($\hat{\mu}+\hat{s}_i$)			
Males	104	61.9	218.3
Females	104	58.7	155.5
Over-all ($\hat{\mu}$)	208	60.3	186.9
Heterosis ($\hat{\mu}+\hat{h}_j$)			
Linebreds	72	59.2	182.9
Crosses	136	61.4	190.9
Linebreds ($\hat{\mu}+\hat{h}_1+\hat{p}_{1k}$)			
AA	22	55.9	175.8
BB	26	66.3	213.8
DD	12	53.7	170.3
HH	12	60.9	171.7
Genic breeding value for crossing ($\hat{\mu}+\hat{h}_2+2\hat{g}_{2k}$)			
A	72	67.4	206.1
B	64	58.6	194.3
D	68	67.0	185.9
H	68	52.6	177.3
Maternal effects ($\hat{\mu}+\hat{h}_2+\hat{m}_{2l}$)			
A	38	59.7	181.1
B	32	62.9	211.7
D	32	55.6	182.1
H	34	67.4	188.7
Crosses ($\hat{\mu}+\hat{h}_2+\hat{g}_{2k}+\hat{g}_{2l}+\hat{d}_{2kl}+\frac{\hat{m}_{2k}+\hat{m}_{2l}}{2}$)			
AB	24	61.6	203.9
AD	22	63.3	186.0
AH	26	63.7	188.2
BD	22	62.1	198.6
BH	18	59.1	194.4
DH	24	58.6	174.3
Reciprocal crosses ($\hat{\mu}+\hat{h}_2+\hat{g}_{2k}+\hat{g}_{2l}+\hat{d}_{2kl}+\hat{r}_{2kl}+\hat{m}_{2l}$)			
AB	12	59.2	184.1
AD	12	64.3	182.7
AH	14	61.6	191.7
BA	12	64.0	223.7
BD	12	64.6	211.5
BH	8	57.3	203.3
DA	10	62.2	189.3
DB	10	59.7	185.7
DH	12	50.5	166.3
HA	12	65.7	184.7
HB	10	61.0	185.5
HD	12	66.7	182.3

of differences among the means such as that described by DUNCAN (1955) and extended by KRAMER (1957) was not made.

The highly significant difference between sexes in favor of the males is in agreement with previous findings (KING 1915; LIVESAY 1930; BECKER 1957).

Heterosis was measured as the comparison of inbreds with line crosses. A more desirable comparison would have been between line crosses and the outbred populations from which the inbred lines were derived. This comparison would indicate whether the superiority of the line crosses was merely the recovery from inbreeding degeneration or a true superiority. Heterosis was not quite significant at 28 days but was significant at 70 days. LIVESAY (1930) made two crosses among three inbred lines of rats and observed heterosis for body weight at 30, 50, 70, 90, and 150 days. The relative superiority of the line crosses increased with increasing age. In the present data, maternal effects may have been relatively greater at 28 days and tended to mask the effect of heterosis. It is also possible that heterosis affects general postnatal growth rate and that differences become more pronounced with increasing age. DICKERSON, LUSH, and CULBERTSON (1946) compared single-cross with inbred litters by the same sire among 11 inbred lines of swine and found that with respect to weight the line crosses tended to exceed the inbreds to a greater extent with increasing age.

Estimates of the heterosis achieved in each cross separately and the individual tests of significance are presented in Table 3. Although these tests of significance

TABLE 3

Heterosis as exhibited by each cross separately

Cross	28-day weight			70-day weight		
	Mean for linebreds	Mean for crosses	Difference	Mean for linebreds	Mean for crosses	Difference
AB	61.1	61.6	.5	194.8	203.9	9.1
AD	54.8	63.3	8.5**	173.0	186.0	13.0
AH	58.4	63.7	5.3*	173.8	188.2	14.4*
BD	60.0	62.1	2.1	192.0	198.6	6.6
BH	63.6	59.1	—4.5	192.8	194.4	1.6
DH	57.3	58.6	1.3	171.0	174.3	3.3
Over-all	59.2	61.4	2.2	182.9	190.9	8.0*

* Significant at .05 level of probability.
** Significant at .01 level of probability.

are not independent, since the linebreds are each used three times, they are of considerable interest because of the variability in heterosis from cross to cross. The average heterosis for crosses involving line A was 4.8 gms for 28-day weight and 12.2 gms for 70-day weight; corresponding values for the B line were —0.6 gms and 5.8 gms; line D, 4.0 gms and 7.6 gms; and line H, 0.7 gms and 6.4 gms. Hence, line A performed the best in crosses and line B, which was the best inbred, performed the poorest in crosses.

The highly significant differences among linebreds for 28- and 70-day weight was consistent with previous experience with these lines. Line H was 5.0 gms

heavier than line A at 28 days and 4.1 gms lighter at 70 days. This may have reflected a difference in maternal ability. Although only linecross litters were used to measure maternal ability, all dams were inbred, i.e., no linecross females produced litters. Linebred progeny in line H may have received better maternal effects prior to weaning and a compensating effect occurred after weaning. YAO and EATON (1954) attributed a similar observation in inbred strains of rabbits to different postnatal growth rates.

General combining ability effects are highly significant at 28 days, but not significant at 70 days. Preweaning differences in maternal ability and a postweaning compensating effect might also account for these results. HENDERSON (1949) studied single crosses among 12 inbred lines of swine and found that general combining ability accounted for not more than five percent of the variation among line crosses with respect to litter size and weight at birth, 21, 56, and 154 days.

There was no evidence of either specific combining ability or sex-linked effects at either age. HENDERSON (1949) found specific effects (dominance and epistasis) to account for five to 15 percent of the variation among swine crosses. EATON, NEVILLE, and DICKERSON (1950) made the 72 possible crosses among nine inbred lines of mice. They found that specific linecross combination effects were important though not significant for viability and total litter weight, but not for individual mouse weights. It is quite possible that crosses among more rat lines might reveal some specific effects. HENDERSON (1949) found no evidence of sex-linked effects.

Maternal effects were highly significant at 28 and 70 days. BAILEY (1954) using a cross-nursing technique also found maternal effects to be important at these ages. Important maternal effectes have been found in rabbits by VENGE (1950) and by YAO and EATON (1954), and in mice by MARSHAK (1936). HENDERSON (1949), however, found no evidence of maternal effects in single crosses among 12 inbred lines of swine. This latter finding is not inconsistent with the observation of CHAMBERS and WHATLEY (1951) of heterosis in productivity of linecross female swine.

The relation between the least squares constants for maternal effects and general combining ability effects for 28- and 70-day weights is shown in Table 4. These constants are expected to be negatively correlated ($r = -0.57$) because maternal effects and general combining ability effects are highly confounded and the correlation is obtained from the elements of the inverse matrix. The difference between the observed and the expected r may provide some information concerning the genetic correlation between maternal and general combining ability effects. With only four lines the information is indeed meager, but it is of interest to note the difference in the estimate of the genetic correlation from 28- to 70-day weight.

If all crossbred offspring exhibit the same amount of heterosis, the differences among the maternal effects as measured in crossbreds will be the same as expected in linebreds. Also, if the model has properly described the effects in crosses, the

TABLE 4

Correlation between maternal and general combining ability effects

Line	28-day weight			70-day weight		
	Maternal		G C A	Maternal		G C A
A	—1.7	. .	3.0	—9.8	. .	7.6
B	1.5	. .	—1.4	20.8	. .	1.7
D	—5.8	. .	2.8	—8.8	. .	—2.5
H	—6.0	. .	—4.4	—2.2	.	—6.8
Observed r		—.93			—.01	
Expected r		—.57			—.57	
Difference		—.36			+.56	

additively genetic effects as estimated from crosses will provide an unbiased estimate of the transmittible genic effects for linebreds as well as crosses. Hence, the differences between the $\hat{p}_{1k}$ and the $2\hat{g}_{2k} + \hat{m}_{2l}$ will indicate whether non-additive effects are operative within line and/or whether the lines tend to rank differently with respect to maternal effects when linebred or crossbred progeny are being reared. Table 5 compares the $2\hat{g}_{2k} + \hat{m}_{2l}$ with the $\hat{p}_{1k}$ for all four lines and both traits. The difference between the observed and expected linebred con-

TABLE 5

Comparison of the $2\hat{g}_{2k}+\hat{m}_{2l}$ *with the* $\hat{p}_{1k}$

Line	28-day weight			70-day weight		
	$2\hat{g}_{2k}+\hat{m}_{2l}$	$\hat{p}_{1k}$	Difference	$2\hat{g}_{2k}+\hat{m}_{2l}$	$\hat{p}_{1k}$	Difference
A	4.4	—3.3	—7.7**	5.4	— 7.1	—12.5
B	—1.3	7.1	8.4**	24.1	30.9	6.8
D	— .3	—5.5	—5.2	—13.8	—12.6	1.2
H	—2.8	1.7	4.5	—15.7	—11.2	4.5

** Significant at the .01 level of probability.

stant is highly significant for lines A and B at 28 days. The correlation between observed and expected constants is -0.51 at 28 days and $+0.91$ at 70 days.

These results clearly suggest that, for 28-day weight, these lines do rank differently with respect to maternal effects when the dams are rearing crossbred progeny than when they are rearing linebred progeny. In other words, an interaction of maternal effects with mating system is indicated.

Except for the lack of specific effects in these data and their presence in swine, and the presence of maternal effects in these data and their absence in swine, these results are in general agreement with those found in other mammalian species. The data are clearly insufficient to warrant any broad generalizations with respect to hybrid vigor in crosses among inbred lines of rats.

SUMMARY AND CONCLUSIONS

The 16 possible combinations including inbreds and reciprocal crosses were made among four inbred lines of rats. The analytical method provided estimates of the effects of sex, heterosis, lines, general and specific combining ability, sex linkage, and maternal effects on 28- and 70-day body weight. Effects of sex, lines and maternal ability were highly significant at 28 and 70 days. Heterosis was significant at 70 but not at 28 days. General combining ability was highly significant at 28 but not at all significant at 70 days. There was no evidence of specific combining ability or sex linkage effects. An interaction of maternal effects and mating system with respect to 28-day weight was indicated.

LITERATURE CITED

BAILEY, D. W., 1954 The inheritance of maternal influences on growth of the rat. Ph.D. Thesis. University of California Library.

BECKER, W. A., 1957 The genetics of body weight in rats: I. Selection progress and linkage. II. Sources of phenotypic variance. Ph.D. Thesis. University of California Library.

CHAMBERS, D., and J. A. WHATLEY, JR., 1951 Heterosis in crosses of inbred lines of Duroc swine. J. Animal Sci. **10**: 505–515.

DICKERSON, G. E., J. L. LUSH, and C. C. CULBERTSON, 1946 Hybrid vigor in single crosses between inbred lines of Poland China swine. J. Animal Sci. **5**: 16–24.

DICKERSON, G. E., 1952 Inbred lines for heterosis tests? Ch. 21. *Heterosis.* Edited by JOHN GOWEN. Iowa State College Press. Ames, Iowa.

DUNCAN, D. B., 1955 Multiple range and multiple F tests. Biometrics **11**: 1–42.

EATON, N. E., W. E. NEVILLE, and G. E. DICKERSON, 1950 General and specific combining abilities in mouse crosses. J. Animal Sci. (Abst.) **9**: 636–637.

HENDERSON, C. R., 1948 Estimation of general, specific and maternal combining abilities in crosses among inbred lines of swine. Unpublished Ph.D. Thesis. Iowa State College Library. Ames.

1949 Estimation of general, specific and maternal combining abilities in crosses among inbred lines of swine. J. Animal Sci. (Abst.) **8**: 606.

1952 Specific and general combining ability. Ch. 22. *Heterosis.* Edited by JOHN GOWEN. Iowa State College Press. Ames, Iowa.

KING, H. D., 1915 The growth and variability in body weight of the Albino rat. Anat. Record **9**: 751–776.

KRAMER, C. Y., 1957 Extension of multiple range tests to group correlated adjusted means. Biometrics **13**: 13–18.

LIVESAY, E. A., 1930 An experimental study of hybrid vigor on heterosis in rats. Genetics **15**: 17–54.

MARSHAK, A. G., 1936 Growth differences in reciprocal hybrids and cytoplasmic influence on growth in mice. J. Exptl. Zool. **72**: 497–510.

VENGE, O., 1950 Studies of the maternal influence on the birth weight in rabbits. Acta Zool. **31**: 1–148.

YAO, T. S., and O. N. EATON, 1954 Heterosis in the birth weight and slaughter weight in rabbits. Genetics **39**: 667–676.

GENE HOMOLOGIES IN COLUMBIDAE[1]

J. H. STIMPFLING[2] AND M. R. IRWIN

Department of Genetics, University of Wisconsin, Madison, Wisconsin

Received August 21, 1959

THE antigens of the red blood cells of a number of species of pigeons and doves possess serological properties which exhibit varying degrees of interrelationships (IRWIN 1951, 1953). In addition to antigenic specificities shared in common, the respective species of pigeons and doves have been shown to possess specificities to the exclusion of one or more other species. In certain instances, it has been established that the cellular antigens of a particular species, in contrast to the cellular antigens of one or more other species, are polygenically determined (IRWIN, COLE and GORDON 1936; IRWIN 1939; IRWIN and CUMLEY 1947). The application of the methods of both genetics and serology have shown that the species-specific cellular antigens are comprised of several components each of which is inherited as a unit character and is, presumably, determined by a single gene or two or more closely linked genes. A cellular antigen which is inherited as a unit is defined as a *unit antigen*. The unit antigens derived from any one species are, in general, serologically distinct. However, a unit antigen characteristic of a particular species may share serological properties with a unit antigen characteristic of one or more other species.

Following successive backcrosses to *Streptopelia risoria* of the hybrids between *S. chinensis* and *risoria* and selected backcross hybrids, a segregation was observed of nine cellular antigens which were characteristic of *chinensis* in contrast to *risoria* (IRWIN and COLE 1936). The *chinensis* specific antigens were given the respective designations d-1, d-2 . . . etc. Likewise, cellular antigens specific to *S. senegalensis*, *S. humilis*, and *S. orientalis* have been identified by mating to *risoria* the hybrid progeny produced from the crosses of these three species to *risoria* for several consecutive generations (IRWIN and CUMLEY 1947; IRWIN, unpublished data). There are at least nine chromosomes of *senegalensis* that carry a gene or genes determining cellular antigens which differentiate *senegalensis* from *risoria*. The *senegalensis*-specific antigens were designated s-1, s-2 . . . etc.; the unit cellular antigens of *humilis* have been designated hu-1, hu-4 and hu-8, and those of *orientalis* as or-1, or-4 and or-8. Further, it has been shown that there are at least four unit antigens A, B, C and E present on the red blood cells of *Columba guinea* which are contrasted to the cellular antigens of *C. livia* (IRWIN, COLE and GORDON 1936).

[1] From the Department of Genetics (No. 744), Agricultural Experiment Station, University of Wisconsin. This project was supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation, and in part by a grant (E-1643) from the Department of Health, Education and Welfare of the National Institutes of Health, Public Health Service.

[2] Present address: Jackson Memorial Laboratory, Bar Harbor, Maine.

The cellular antigen d-1 of *chinensis* and s-2 of *senegalensis* share antigenic properties (IRWIN and COLE 1940). Both d-1 and s-2 are serologically related to the cellular antigens hu-1, or-1 and E (IRWIN, unpublished data). For reasons of uniformity, the d-1 antigen of *chinensis* and the s-2 antigen of *senegalensis* will be referred to as ch-1 and se-1 respectively. The nomenclature for hu-1, or-1 and E will remain unchanged. Henceforth, the related cellular antigens ch-1, hu-1, or-1, se-1 and E will be considered as members of a system defined as group-1.

This paper is concerned with an analysis of the genetic and serological properties of the different cellular antigens belonging to the group-1 system.

MATERIALS AND METHODS

The serological technics employed here were similar to those which have been described previously in publications from this laboratory (IRWIN and COLE 1936; IRWIN, COLE and GORDON 1936; IRWIN 1939; MILLER and BRYAN 1953). The hemagglutination technic was used throughout these tests. Antisera were obtained after injecting into rabbits the washed red blood cells from backcross hybrids possessing one or another of the respective cellular antigens ch-1, hu-1, or-1, se-1 or E; other antisera were prepared in the same manner against cells from each of the species *chinensis*, *humilis*, *orientalis*, *senegalensis* and *guinea*.

The chromosomes bearing the genes for the unit cellular antigens ch-1, hu-1, or-1 and se-1, respectively, have been transferred from the particular parental species to backcross hybrids whose blood cells are serologically indistinguishable from the cells of *risoria*, except for the presence of one or another of the group-1 cellular antigens. Consequently, antisera prepared against the blood cells of the backcross hybrids may contain agglutinins against all the cellular antigens of *risoria* as well as agglutinins specific for a particular group-1 antigen. Agglutinating reagents specific for the cells of ch-1, hu-1, or-1 and se-1 backcross hybrids were prepared by absorbing their respective antisera with the pooled cells of *risoria*. The cellular antigen E of *guinea* has also been isolated in backcross hybrids whose cells are serologically indistinguishable from the cells of *livia*, except for the presence of the antigen E. Specific reagents for E were prepared by absorbing anti-E sera with cells from *livia*.

The red blood cells of *livia* and *risoria* undoubtedly share many antigens, but *livia* shares no known antigenic specificities with ch-1, hu-1, or-1 or se-1 that it does not also share with *risoria*. As a consequence, specific reagents for the cellular antigens ch-1, hu-1, or-1 and se-1 can be obtained by absorbing their respective antisera with pooled cells from *risoria* and *livia*. The pooling of cells from the two species, *risoria* and *livia*, was done whenever possible. It was necessitated by occasional shortages of *risoria* cells, and also provided an added advantage of absorbing from the antisera antibodies which might differentiate individuals within either species. Some anti-E sera, following absorption with cells from *livia*, weakly agglutinate the cells of *risoria;* therefore, it was necessary to absorb the anti-E sera with cells from both *risoria* and *livia*.

Although the cellular antigen se-12 (formerly s-12) of *senegalensis* was identified as a separate entity in the first backcross generation, it now seems probable that se-1 and se-12 are contrasting characters in *senegalensis*, with common as well as differentiating antigenic specificities. Antisera prepared against the cells of se-1 backcross hybrids and the cells of *senegalensis* often contain antibodies reactive with the cells of backcross hybrids possessing se-12. In order that these tests would be limited to the relationship of se-1 to the other antigens of group-1, and not be complicated by antibodies directed to se-12, all se-1 antisera were absorbed with cells carrying se-12 as well as those of *risoria* and *livia*.

RESULTS

Following absorption with cells from *risoria* and *livia* of the appropriately diluted antisera which had been prepared against the cells of the respective species and backcross hybrids, each of the absorbed antisera was tested against the cells of backcross hybrids containing one or another of the cellular antigens ch-1, hu-1, or-1, se-1 or E; the reactions of the absorbed antisera were also determined for cells from the species *chinensis*, *humilis*, *orientalis*, *senegalensis* and *guinea*. The agglutination reactions of the absorbed fluids are given in Table 1.

From these reactions it may be seen that the reagents prepared from antisera against each of the group-1 antigens are cross-reactive with the cells of all members of group-1 (upper left quadrant of Table 1) and also, as expected, with the

TABLE 1

Agglutination reactions of absorbed fluids

Cells tested	Antisera									
	ch-1	hu-1	or-1	se-1*	E	*chinensis*	*humilis*	*orientalis*	*senegalensis**	*guinea*
	Absorbed by pooled cells from *S. risoria* and *C. livia*									
risoria	O	O	O	O	O	O	O	O	O	O
livia	O	O	O	O	O	O	O	O	O	O
se-12	O	O	O	O	O	O	O	O	O	O
ch-1	C†	C	++	C	C	C	C	C	C	C
hu-1	++	C	C	++	C	C	C	C	C	++
or-1	C	C	C	C	C	C	C	C	C	C
se-1	C	C	C	C	C	C	C	C	C	C
E	C	+	C	++	C	C	C	C	++	C
chinensis	C	C	C	C	C					
humilis	C	C	++	C	C					
orientalis	C	C	C	C	C					
senegalensis	C	C	C	C	C					
guinea	C	+	++	+	C					

* Absorbed by cells from backcross hybrids possessing the se-12 cellular antigen, in addition to the pooled cells of *risoria* and *livia*.

† C=complete agglutination; ++=almost complete agglutination; +=weak agglutination; O=no agglutination at a serum dilution of 1:30 or greater.

respective species from which each member of the group-1 antigens was derived (lower left quadrant of Table 1). Reciprocally, the antisera to each of these parental species, following absorption with the cells of *risoria* and *livia*, were also reactive with each of the different members of the group-1 antigens (upper right quadrant of Table 1). Individuals have been observed in *senegalensis* which lack se-1 (IRWIN and COLE 1945), but only those carrying se-1 have been used in these experiments.

Since antisera prepared against the parental species, after absorption with the cells of *risoria* and *livia*, contain antibodies specific for antigens other than those of group-1, the agglutination of cells from the homologous and heterologous species by the respective absorbed antisera cannot be interpreted in terms of group-1 reactions or cross reactions. Consequently, the lower right quadrant of Table 1 has been left vacant.

The results presented in Table 1 show that the cellular antigens ch-1, hu-1, or-1, se-1 and E share serological properties among themselves and therefore with the corresponding antigens found on the cells of the parental species. The complexity of these interrelationships cannot be deduced from the data given in Table 1. However, the observation that cells from backcross hybrids possessing one or another of the group-1 antigens differ appreciably in the intensity of their agglutination reactions with respect to a particular group-1 reagent suggests the absence of serological equivalence, e.g., the hu-1 reagent agglutinates very strongly cells which possess the homologous group-1 antigen but weakly agglutinates the cells of E backcross hybrids and *guinea*. A more detailed serological analysis of the interrelationships within the group-1 system will be presented in a subsequent paper.

Since the backcross hybrids possessing the cellular antigens ch-1, hu-1, or-1 and se-1, respectively, were derived from matings in which the common parent was *risoria*, it was considered that *risoria* might possess cellular antigens which were antithetically related to the cellular antigens of one or more members of group-1. It has not been possible, for technical reasons, to make the necessary matings required for the separation and identification of the unit cellular antigens of *risoria*. However, in 1953, MILLER and BRYAN described a method for the detection, on the cells of *livia* and of backcross hybrids from the mating of *guinea* × *livia*, of antigens antithetically related to the unit cellular antigens possessed by *guinea* in contrast to *livia*. The procedures followed in the experiments described here are essentially the same as those reported by MILLER and BRYAN.

If *risoria* possess a gene or genes allelic to the genes which determine the presence of one or more members of the group-1 cellular antigens, and the gene or genes of *risoria* determine the presence of an antigen on the red blood cells in contrast to a particular group-1 cellular antigen, then derivatives of backcross hybrids which are homozygous for any one of the group-1 cellular antigens will lack the cellular antigen of *risoria*. The blood cells of the derivatives of the backcross hybrids which are homozygous for the gene or genes which determine a

particular group-1 cellular antigen should, in other respects, be serologically indistinguishable from those of *risoria*.

If the preceding suppositions are correct, it should be possible to demonstrate the presence of the cellular antigen of *risoria*, which is antithetically related to the contrasting group-1 cellular antigen, with reagents prepared by absorbing anti-*risoria* serum with cells of hybrids which are homozygous for genes determining the presence of the particular group-1 cellular antigen. Absorption of an anti-*risoria* serum with the cells of any group-1 homozygote should remove all *risoria*-specific agglutinins, except those which are specific for the *risoria* cellular antigen antithetically related to the contrasting group-1 antigen. Such absorbed fluids will agglutinate the blood cells of *risoria* and the corresponding group-1 heterozygotes.

The cellular antigens of *risoria* which are presumed to be antithetically related to the antigens ch-1, hu-1, or-1 and se-1, will be given tentatively the corresponding designations of ch-1′, hu-1′, or-1′ and se-1′.

All progeny produced by matings *inter se* of group-1 backcross hybrids will belong to one or another of the three classes group-1/group-1, group-1/group-1′ or group-1′/group-1′. The offspring will fall into one or another of the three classes with the frequencies expected of a monohybrid cross. Cells from individuals of the class group-1′/group-1′ will be serologically indistinguishable from the cells of *risoria*. However, cells from individuals of the two classes group-1/group-1 or group-1/group-1′ will share in common their agglutinability by all group-1 reagents since they are homozygous and heterozygous, respectively, for the genes which determine the group-1 cellular antigens. The two latter classes may be differentiated further with regard to their respective capacities to absorb agglutinins from an anti-*risoria* serum, as previously described.

To test the hypothesis that the group-1 cellular antigens are antithetically related to one or more cellular antigens in *risoria*, cells from group-1 positive progeny produced by *inter se* matings of heterozygotes of ch-1, or-1 and se-1 were used from individual birds to absorb an anti-*risoria* serum. As expected, the cells from some group-1 positive progeny produced by the above matings had the capacity to absorb completely from the anti-*risoria* serum the agglutinins specific for the cells of *risoria;* the birds from which these cells had been obtained were regarded as putative heterozygotes, i.e., ch-1/ch-1′, or-1/or-1′ and se-1/se-1′, respectively. The cells from the remaining group-1 positive progeny produced by the above matings, when used for absorption, failed to remove all agglutinins from the anti-*risoria* serum which were specific for the cells of *risoria;* the birds from which these cells had been obtained were considered to be putative homozygotes, i.e., ch-1/ch-1, or-1/or-1 and se-1/se-1. The cells of the respective classes of homozygotes were pooled and used to absorb the anti-*risoria* serum. Each of the three absorbed fluids was presumed to be a reagent which contained agglutinins for the cellular antigens of *risoria* antithetically related to the cellular antigens ch-1, or-1 and se-1, respectively.

The reagents specific for ch-1′, or-1′ and se-1′ were tested against the cells of

a number of individuals and their corresponding progeny. The respective reagents for ch-1 and ch-1′ were tested with the cells of the progeny from matings involving either or both of these cellular characters, with results as shown in the first three rows of Table 2. The matings were ch-1/ch-1 × ch-1/ch-1, ch-1/ch-1′ × ch-1/

TABLE 2

Tests for reagent specificity

Type of mating	No. of matings	No. of progeny	Number of progeny per class		
			1/1	1/1′	1′/1′
ch-1/ch-1 × ch-1/ch-1	1	11	11	0	0
ch-1/ch-1′ × ch-1/ch-1′	6	39	12	20	7
ch-1/ch-1′ × *risoria*	6	31	0	16	15
or-1/or-1 × or-1/or-1′	5	17	9	8	0
or-1/or-1′ × or-1/or-1′	9	33	5	24	4
or-1/or-1′ × *risoria*	7	16	0	7	9
se-1/se-1 × se-1/se-1′	2	33	16	17	0
se-1/se-1 × *risoria*	1	10	0	10	0
se-1/se-1′ × *risoria*	7	45	0	23	22

ch-1′ and ch-1/ch-1′ × *risoria* (ch-1′/ch-1′); the progeny produced from these matings appeared in the expected classes with approximately the frequency expected if ch-1 and ch-1′ are antithetically related antigens. The results of the tests involving or-1 and or-1′ are given in the second group of three rows in Table 2. In this instance the matings were or-1/or-1 × or-1/or-1′, or-1/or-1′ × or-1/or-1′ and or-1/or-1′ × *risoria* (or-1′/or-1′); these matings produced progeny which appeared in the appropriate classes with approximately the frequencies expected if or-1 and or-1′ have an antithetical relationship. Matings which involved se-1 and se-1′ provided the data which are included in the last three rows of Table 2. In this case the parental types were se-1/se-1 × se-1/se-1′, se-1/se-1 × *risoria* (se-1′/se-1′) and se-1/se-1′ × *risoria*. Here, too, the progeny appeared in the expected classes with frequencies consistent with those expected if se-1 and se-1′ are antithetically related cellular antigens. Although the number of progeny produced by any particular class of mating is not large, it is important to note that no exceptions to the expected have appeared. The data presented in Table 2 validate the supposition that there are present on the blood cells of *risoria* one or more cellular antigens which bear an antithetical relationship to the cellular antigens of each of three members of group-1, i.e., ch-1, or-1 and se-1.

Table 2 gives the results of tests performed on the cells of 235 progeny produced by the various matings. With the exception of 75 individuals, all progeny were tested with the three group-1′ reagents, anti-ch-1′, anti-or-1′ and anti-se-1′. When the three reagents were used at a serum dilution of 1:60, they paralleled each other with respect to their agglutinating capacity, i.e., they either agglutinated

or failed to agglutinate a particular test cell. Therefore, it is concluded that the group-1′ reagents contained agglutinins specific for a single antigen or antigenic complex found on the cells of *risoria*. The cellular antigen of *risoria* which has been demonstrated to be antithetically related to the cellular antigens ch-1, or-1, and se-1, respectively, may now be called ri-1. Further, if ch-1, or-1 and se-1 are antithetically related to ri-1, they are presumably related in the same way to each other.

Further confirmation of the antithetical relationship of or-1 and se-1 has been obtained from two matings of the type: or-1/or-1 × se-1/ri-1. Eight progeny were produced by these matings; all carried or-1 as expected, and there were four with ri-1 (or-1/ri-1) and four with se-1 (or-1/se-1). There were also 11 progeny from two matings of ch-1/se-1 × ri-1/ri-1 *(risoria)*. Ten of these possessed ch-1/ri-1, and one carried se-1/ri-1. Two matings to *risoria* of backcross hybrids carrying ch-1/or-1 resulted in 20 offspring with ch-1 and nine with or-1. The cells of the progeny were not tested with the reagent for ri-1; all would be expected to possess it.

It was stated previously that se-12 appears to be a contrasting character to se-1 in *senegalensis*. Evidence of the antithetical nature of se-12 to ch-1 of *chinensis* has been obtained from matings of ch-1/ri-1 × se-12/ri-1, which gave progeny as follows: there were eight with both ch-1 and se-12, four with ch-1, seven with se-12, and three with neither. The reagent for ri-1 was not available at the time for testing the cells of these progeny, but the cells of all the progeny except those carrying ch-1/se-12 should have been reactive. Two matings to *risoria* were made of backcross hybrids carrying ch-1/se-12. Of the 18 progeny, there were 10 with ch-1 and eight with se-12. The reagent for ri-1 was not available to test these cells. The results are in agreement with the expected if the various characters of group-1 are antithetical to each other, and if se-12 is one of this group.

In addition to its serological relationship to group-1, the inclusion of hu-1 in the group-1 system is supported by the following evidence: Among four progeny produced from the mating F_1-*humilis/risoria* × F_1-*humilis/risoria* the cells of one individual reacted with the group-1′ reagents, only, and was presumed to be ri-1/ri-1; the cells of two individuals reacted with both group-1 and group-1′ reagents and were regarded as hu-1/ri-1; the cells of the remaining bird were agglutinated by the group-1 reagents but were not agglutinated by the group-1′ reagents and must have been hu-1/hu-1. From a mating between two backcross hybrids hu-1/ri-1 × ch-1/ri-1 there was produced a single offspring, the cells of which possessed the cellular antigens hu-1 and ch-1 (hu-1/ch-1), but which lacked the antigen ri-1. Although the genetic evidence for including hu-1 in the group-1 system is limited, the available data are compatible with the assumption that hu-1 is antithetically related to ch-1, or-1, ri-1 and se-1. It is probable that tests on additional progeny from appropriate matings will justify placing the hu-1 cellular antigen in the group-1 system.

It has not, as yet, been possible to obtain genetic evidence for the antithetical relationship of the cellular antigen E to the other members of group-1. The inclu-

sion of E in the group-1 system must be based upon the demonstrated serological relationship of E to the cellular antigens ch-1, hu-1, or-1 and se-1.

DISCUSSION AND CONCLUSION

Based on the consideration that the cross-reactivity of cellular antigens reflects structural similarity of a greater or less degree, the cellular antigens described in this paper are regarded as structural homologues. Further, it is considered plausible that the gene or genes which determine the structural properties of cross-reactive cellular antigens in related species are themselves structurally related to an extent which varies with the degree of the serological relationship of their antigenic products.

It has been proposed that homologous genes in various species be defined as those which behave as alleles when present in a common genetic background (SPENCER 1949). This criterion is in general not subject to experimental verification, except in those species capable of producing fertile hybrid offspring. A less direct approach is to make a comparative study of related structures and from these investigations attempt to deduce the structural correspondence of the causative genes (STURTEVANT and NOVITSKI 1941; SILOW 1941; HARLAND 1936; STEPHENS 1951). The majority of the studies have been concerned with a comparative examination of the terminal products of putative alleles in related species; however, in some cases, it has been possible to apply the genetic criterion of allelism. The data presented in this paper were obtained by the application of both criteria. It has been demonstrated that the genes which determine certain members of group-1, i.e., ch-1, or-1, ri-1, se-1 and probably hu-1, are borne on chromosomes exhibiting an antithetical relationship to each other. Consequently, it is presumed that the antithetically related chromosomes are related as homologues. Although it is possible that the gene or genes which determine the respective cellular antigens ch-1, hu-1, or-1, ri-1 and se-1 are nonallelic and that their disposition on homologous chromosomes in the various species from which they have been derived is fortuitous, the data are more simply explained by assuming that the causative genes are alleles. It is proposed that the occurrence in other related species of cellular antigens exhibiting serological affinities to group-1 is at least suggestive evidence that these species also possess genes which are allelic to the genes which determine the respective members of the group-1 system. The inability to demontrate a group-1 antigen in a related species need not be interpreted as indicating the absence of the corresponding gene homologue in that species. Such an instance could arise if the homologous gene determined a cellular antigen with a specificity or specificities not detectable by available group-1 reagents or if the homologous gene failed to determine the presence of any cellular antigen. The absence of an antigenic relationship between the cellular antigen ri-1 and the cellular antigens ch-1, hu-1, or-1 and se-1 arises, of course, from the procedure used to prepare reagents specific for the respective antigens ch-1, hu-1, or-1 and se-1. The fact that at least one anti-E serum, following absorption with the pooled cells of *livia*, retained the ability to agglutinate weakly the cells of *risoria* suggests that E may share serological properties with ri-1, however, this

need not be the case. Subsequent attempts to duplicate the reagent were not successful, presumably because the *risoria*-specific agglutinins were unstable and had been lost from the antiserum.

Previous studies have shown that the cellular antigen E of *guinea* is antithetically related to E′ of *livia* (MILLER and BRYAN 1953). If either E or E′ is shown to be genetically related to the cellular antigens ch-1, hu-1, or-1, ri-1 or se-1 this would constitute evidence that both E and E′ are antithetically related to the group-1 system. Evidence could be obtained from the mating of an F_1-*livia/risoria* (E′/ri-1) to a backcross hybrid homozygous for a group-1 cellular antigen, e.g., ch-1/ch-1. The progeny from this mating will share the antigen ch-1, while one half of the progeny will possess E′ and the remaining one half will carry ri-1. If E′ and ri-1 are determined by alleles they will appear as mutually exclusive in the progeny; if they are loosely linked or located on independently segregating chromosomes, E′ and ri-1 will not be mutually exclusive. Unfortunately, the F_1-*livia/risoria* exhibits low fertility and the required progeny may be difficult to obtain.

If ri-1 is antigenically related to E′, ri-1 agglutinins will be expected to occur in at least some anti-E′ reagents which are prepared by absorbing an anti-*livia* serum with the cells of backcross hybrids homozygous for E. The occurrence of anti-ri-1 agglutinins in an anti-E′ reagent will be supporting evidence that both E and E′ are antithetically related to the other members of the group-1 system which have been considered here.

SUMMARY

Backcross hybrids were obtained by mating to *Streptopelia risoria* the species hybrids and selected backcross hybrids from matings of *S. chinensis*, *S. humilis*, *S. orientalis*, and *S. senegalensis* to *risoria*. Similarly, backcross hybrids were obtained by mating to *Columba livia* the species hybrids and selected backcross hybrids from matings of *C. guinea* to *C. livia*. Cellular antigens of the erythrocytes which distinguish each of these species from *risoria* and *livia* are determined either by single genes or by two or more closely linked genes. The following antigenic characters were considered: ch-1 of *chinensis*, hu-1 of *humilis*, or-1 of *orientalis*, ri-1 of *risoria*, se-1 of *senegalensis* and E of *guinea*. The six unit antigens have been collectively designated as belonging to the group-1 system.

Following appropriate absorptions and hemagglutination tests, it was demonstrated that the cellular antigens ch-1, hu-1, or-1, se-1 and E constitute a serologically cross reactive system. As a consequence of a combination of genetic and serological tests, it was shown that antigens ch-1, or-1, se-1 and probably hu-1 are determined by genes exhibiting an antithetical relationship to the gene or genes which determine the formation of the ri-1 cellular antigen of *risoria*. It was concluded that since ch-1, hu-1, or-1 and se-1 are antithetically related to ri-1, they are also antithetical to each other. Other genetic evidence is presented which confirms this hypothesis. The inclusion of E into group-1 rests almost entirely upon the serological relationship of E to the other members of group-1 which have been described here.

LITERATURE CITED

HARLAND, S. C., 1936 The genetical concept of the species. Biol. Rev. Cambridge Phil. Soc. **11**: 83–112.

IRWIN, M. R., 1939 A genetic analysis of species differences in Columbidae. Genetics **24**: 709–721.

1951 *Genetics in the 20th Century*. Chap. 10. MacMillan Co. New York.

1953 Evolutionary patterns of antigenic substances of the blood corpuscles in Columbidae. Evolution **7**: 31–50.

IRWIN, M. R., and L. J. COLE, 1936 Immunogenetic studies of species and species hybrids in doves, and the separation of species-specific substances in the backcross. J. Exptl. Zool. **73**: 85–108.

1940 Further studies of the interrelationships of cellular characters in Columbidae. Genetics **25**: 326–336.

1945 Immunogenetic studies of cellular antigens: individual differences between species hybrids. Genetics **30**: 439–447.

IRWIN, M. R., L. J. COLE, and C. D. GORDON, 1936 Immunogenetic studies of species and species-hybrids in pigeons, and the separation of species-specific characters in backcross generations. J. Exptl. Zool. **73**: 285–308.

IRWIN, M. R., and R. W. CUMLEY, 1947 A second analysis of antigenic differences between species in Columbidae. Genetics **32**: 178–184.

MILLER, W. J., and C. R. BRYAN, 1953 Serological differentiation of the homozygotes and the heterozygotes in backcross birds following a species cross in Columbidae. Proc. Natl. Acad. Sci. U. S. **39**: 407–412.

SILOW, R. A., 1941 The comparative genetics of *Gossypium anomalum* and the cultivated Asiatic cottons. J. Genet. **42**: 259–358.

SPENCER, W. P., 1949 *Genetics, Paleontology and Evolution*. Chap. 3. Princeton University Press. Princeton, New Jersey.

STEPHENS, S. G., 1951 Evolution of the gene. "Homologous" genetic loci in Gossypium. Cold Spring Harbor Symposia Quant. Biol. **16**: 131–157.

STURTEVANT, A. H., and E. NOVITSKI, 1941 The homologies of the chromosome elements in the genus Drosophila. Genetics **26**: 517–541.

GENETICS SOCIETY OF AMERICA

EXECUTIVE COMMITTEE

President, JAMES F. CROW — *Vice-President*, H. BENTLEY GLASS

Past Presidents, C. P. OLIVER, KARL SAX

Secretary, W. L. RUSSELL
Oak Ridge National Lab.
Oak Ridge, Tenn.

Treasurer, DREW SCHWARTZ
Oak Ridge National Lab.
Oak Ridge, Tenn.

"The Genetics Society of America is organized to provide facilities for association and conference among students of heredity and for the encouragement of close relationship between workers in genetics and those in related subjects. All persons actively interested in any field of genetics shall be eligible to active membership. Candidates for membership must be recommended by two members of the Society. Members shall be elected by the Executive Committee." —Constitution, Article I.

Any one interested in joining the Society should write to the Secretary.

The Genetics Society publishes annually the Records of the Society containing the program and abstracts of the Annual Meeting and other items of interest to members, including a membership list at periodic intervals. Total membership of the Society was 1117 in May, 1959. Copies of the Records may be obtained from the Treasurer for $1.00. The Genetics Society has a representative on the Editorial Board of GENETICS, Tracy M. Sonneborn.

SUSTAINING MEMBERS

The Genetics Society of America established in 1949 a new type of membership, Sustaining Members. The constitution provides that "All organizations interested in any field of genetics shall be eligible to sustaining membership." The dues for sustaining members are $50.00 a year.

Money contributed by Sustaining Members is not used for running expenses of the Society, but is used for special purposes as decided by the Executive Committee. At the meeting of the Executive Committee on August 31, 1959, $500.00 was allocated to GENETICS from the Sustaining Membership Fund.

The following organizations became sustaining members of the Society in the years indicated:

Animal Breeding Consultants
Rt. 1, Box 97B.
Livermore, California—1958
Associated Seed Growers
New Haven, Conn.—1950
Bear Hybrids Corn Co.
Decatur, Ill.—1950
Bristol Laboratories, Inc.
P. O. Box 657
Syracuse 1, New York—1957
W. Atlee Burpee Co.
Philadelphia 32, Pa.—1950
Colonial Poultry Farms, Inc.
Pleasant Hill, Missouri—1958
DeKalb Hybrid Seed Co.
DeKalb, Ill.—1949
Ferry-Morse Seed Co.
Detroit 31, Mich.—1949
Funk Brothers Seed Co.
Bloomington, Ill.—1950
Ghostley's Poultry Farm
Anoka, Minnesota—1956
Greenwood Seed Company
Greenwood Farms
Thomasville, Georgia—1959
Kimber Farms, Inc.
Niles, California—1953
Moews Seed Co.
Granville, Ill.—1949
Nicholas Turkey Breeding Farms
Sonoma, California—1958
Nichols Poultry Farm
Kingston, N. H.—1950
Northrup King & Co.
Minneapolis 15, Minn.—1957
Pfister Associated Growers, Inc.
Aurora, Ill.—1957
Pioneer Hi-Bred Corn Co.
Johnston, Iowa—1949

This page is donated by GENETICS to the GENETICS SOCIETY OF AMERICA. The society and the journal are separate organizations with a common bond of interest in furthering the science of heredity.

INFORMATION FOR CONTRIBUTORS

(Authors should read New Policies. GENETICS **45**:7–9, 1960 for details)

GENETICS accepts original contributions in genetics proper, or in any scientific field of primary genetic interest.

Ordinarily, manuscripts will appear in print in the order they were received, but *Short* and *Long* papers will be processed on different printing schedules with the short papers appearing at an accelerated rate. *Short* papers can be no longer than *four* printed pages; they must conform to the standards and follow the form required for regular papers. Long papers will be limited to *20* printed pages. A paper longer than 20 pages will be published only by special vote of the Editorial Board and the author will be expected to bear the costs of the additional pages.

An author must submit to the Editors **two** copies of a manuscript, **typewritten** with **double spacing** throughout. All manuscripts must conform to the general usage in GENETICS particularly in regard to references to literature, arrangement of "Literature Cited" and inclusion of a "Summary". A symbol (gene, for example) should be defined or identified the first time it is used. Excessive footnotes should be avoided. Footnotes to text statements, if necessary, can usually be included in the text parenthetically. Tables must be typewritten on separate pages with double spacing throughout, arranged to journal page size ($5 \times 7\frac{3}{8}$), and in a form acceptable to the journal and suitable for the printer to set. (See New Policies, GENETICS **45**:7–9, 1960.) Legends for figures and plates are to be typewritten, double spaced, on separate pages. Material for figures should be original drawings (clear photographs usually are satisfactory for line drawings) and should be of a size permitting slight reduction for the printed page. Illustrations should be mounted in final form for engraving. Photographs and drawings substantially larger than a typewritten page are likely to be damaged in the mail and should not be sent. Reviewing of manuscripts is facilitated if additional photographic copies of figures are sent. Chemical structural formulas, complex mating-type charts, and other sketches must be in form that can be photographed. (Clear photographs are acceptable.) Complex mathematical formulas are preferred in the form of reproducible photographs; otherwise they must be typewritten. (See GENETICS **45**:7–9, 1960.)

The manuscript of a published paper will not be returned unless the author so requests.

Galley proof will be sent to authors; page proof will not be sent. Authors should leave forwarding directions, if necessary, or should make other arrangements to have the galley proof corrected promptly. Send corrected proof to the Editors.

Reprints are to be ordered directly from the Editors at the time galley proof is returned. The author will be billed in accordance with a schedule of charges appearing on the reprint order blank. Copies of the schedule are sent to the author along with galley proof.

The *Galton and Mendel Memorial Fund* established in 1923 from donations of biologists and other persons interested in the progress of biological discovery is available to be applied toward the cost of reproducing illustrations and of printing expensive tables and formulas.

Manuscripts and correspondence should be addressed to the Editors of GENETICS, Experimental Science Building 122, University of Texas, Austin 12, Texas.

CONTENTS OF PRECEDING ISSUES

NOVEMBER, 1959, PART 2

New Policies for Genetics.

DEMARINAS, F., The nature of asymmetry and variability in the double bar-eyeless^2 Drosophila.

ROBERTSON, FORBES W., Studies in quantitative inheritance. XIII. Interrelations between genetic behavior and development in the cellular constitution of the Drosophila wing.

WEBER, E., The genetical analysis of characters with continuous variability on a Mendelian basis. I. Monohybrid segregation.

COCKERHAM, C. CLARK, Partitions of hereditary variance for various genetic models.

FAHMY, O. G., and MYRTLE J. FAHMY, Differential gene response to mutagens in *Drosophila melanogaster*.

NANNEY, D. L., Genetic factors affecting mating type frequencies in variety 1 of *Tetrahymena pyriformis*.

PERKINS, DAVID D., New markers and multiple point linkage data in Neurospora.

PERKINS, DAVID D., and CHIYOKO ISHITANI, Linkage data for group III markers in Neurospora.

MALING, BARBARA, Linkage data for group IV markers in Neurospora.

STRICKLAND, WALTER N., DAVID D. PERKINS, and CATHERINE C. VEATCH, Linkage data for Group V markers in Neurospora.

BALBINDER, ELIAS, The genotypic control of kappa in *Paramecium aurelia*, syngen 4, stock 51.

GREEN, M. M., Non-homologous pairing and crossing over in *Drosophila melanogaster*.

VOGT, MARGUERITE, A study of the relationship between karyotype and phenotype in cloned lines of strain HeLa.

NOVEMBER, 1959, PART 3

New Policies for Genetics.

SWAMINATHAN, M. S., and B. R. MURTY, Aspects of asynapsis in plants. I. Random and non random chromosome associations.

SIMMONDS, N. W., and E. HARRISON, The genetics of reaction to pepper vein-banding virus.

DAVIS, ROWLAND, Asexual selection in *Neurospora crassa*.

JAMES, ALLEN P., The spectrum of severity of mutant effects. I. Haploid effects in yeast.

PARSONS, P. A., Genotypic-environmental interactions for various temperatures in *Drosophila melanogaster*.

CLAYBERG, CARL D., Cytogenetic studies of precocious meiotic centromere division in *Lycopersicon esculentum* Mill.

CLARKE, C. A., and P. M. SHEPPARD, The genetics of *Papilio dardanus*, Brown. I. Race *cenea* from South Africa.

KRIMBAS, COSTAS B., Comparison of the concealed variability in *Drosophila willistoni* with that in *Drosophila prosaltans*.

MORPURGO, G., and G. SERMONTI, Reactivation by manganous chloride of spores inactivated by nitrogen mustard.

JANUARY, 1960

CASPARI, ERNST W., Richard B. Goldschmidt.

New Policies for Genetics.

MIYAKE, T., Mutator factor in *Salmonella typhimurium*.

GREEN, M. M., Double crossing over or gene conversion at the white loci in *Drosophila melanogaster*?

ASHMAN, R. B., Stippled aleurone in maize.

MILKMAN, ROGER D., The genetic basis of natural variation. I. Crossveins in *Drosophila melanogaster*.

HASHIMOTO, KAZUO, Streptomycin resistance in *Escherichia coli* analyzed by transduction.

RHYNE, CLAUDE L., Leaf lobation in Gossypium amphidiploids as determined by leaf-shape genes of Gossypium diploid species.

LEWIS, C. F., and T. R. RICHMOND, The genetics of flowering response in cotton. II. Inheritance of flowering response in a *Gossypium barbadense* cross.

HINTON, CLAUDE W., and JOHN C. LUCCHESI, A cytogenetic study of crossing over in inversion heterozygotes of *Drosophila melanogaster*.

STALKER, HARRISON D., Chromosomal polymorphism in *Drosophila paramelanica* Patterson.

PETERSON, PETER A., The pale green mutable system in maize.

SINGLE NUMBERS $1.50 ANNUAL SUBSCRIPTION $12.00

obtainable from

GENETICS, INC., BUSINESS OFFICE, EXPERIMENTAL SCIENCE BLDG. 122,
UNIVERSITY OF TEXAS, AUSTIN 12, TEXAS